

Locust brain

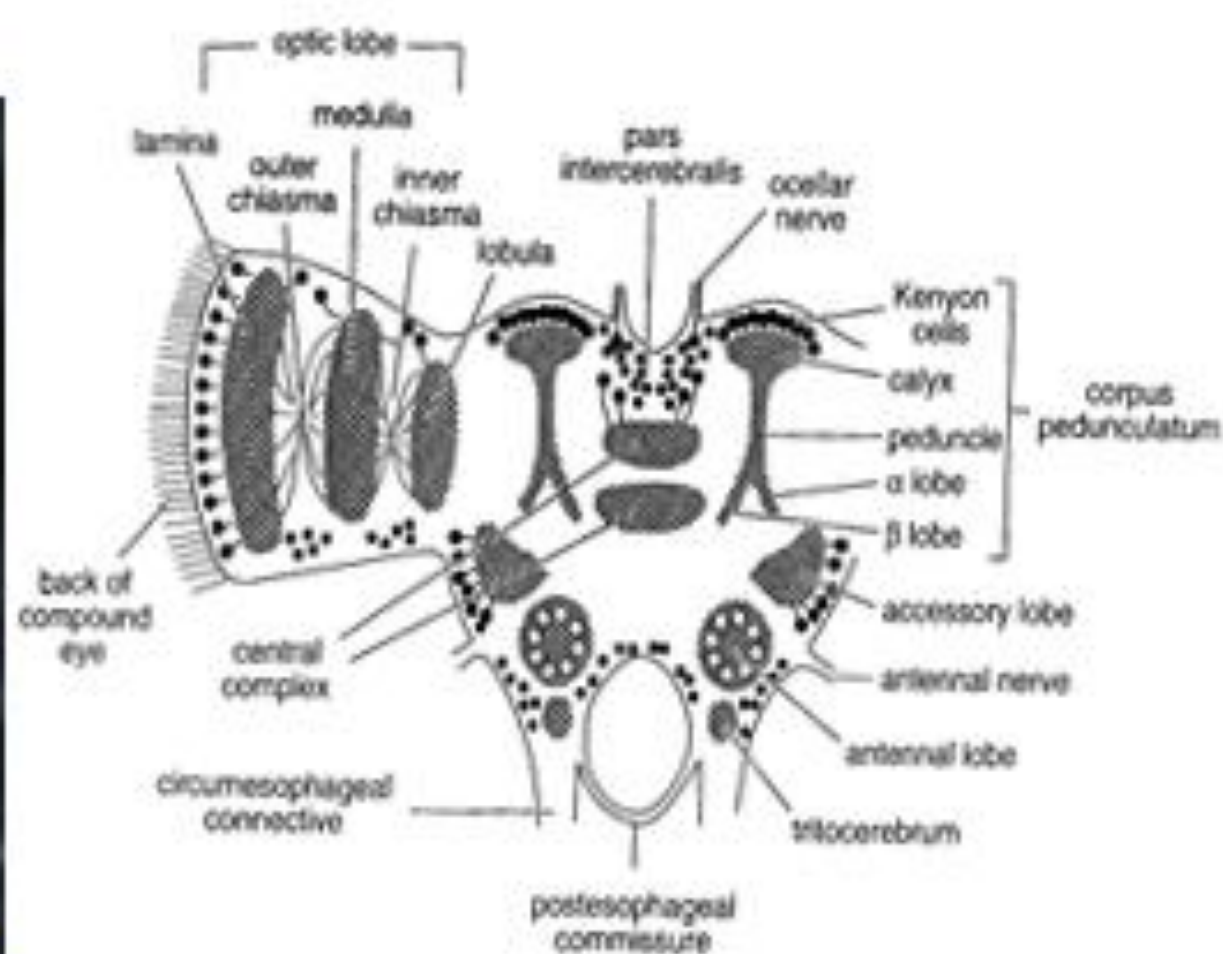
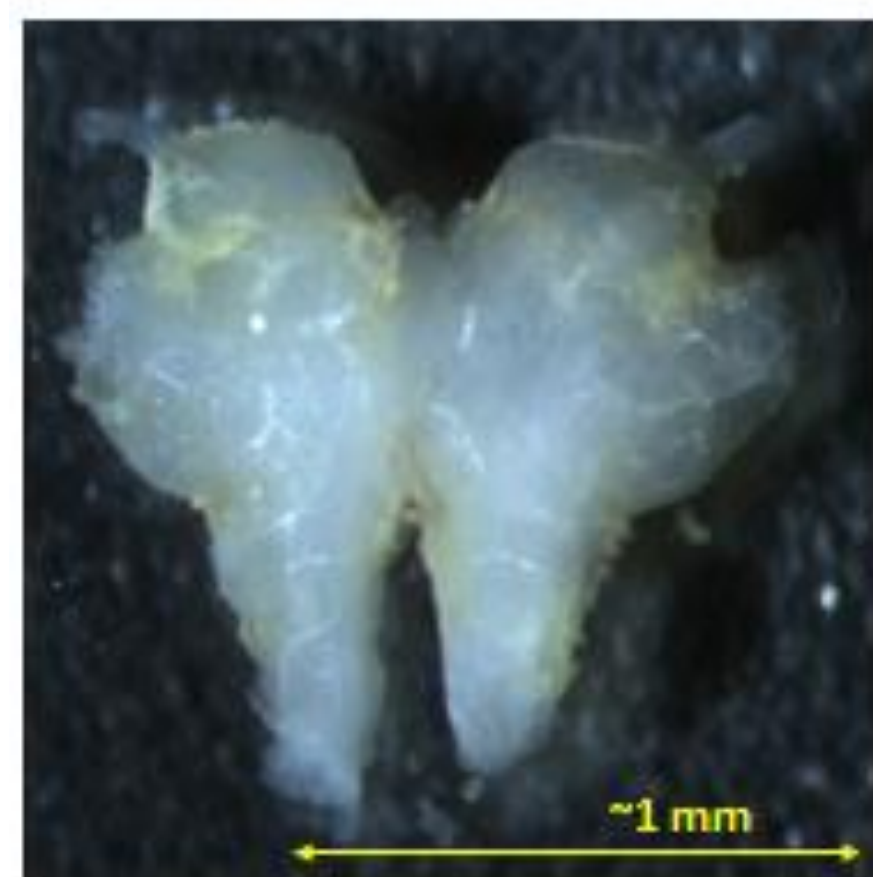


Figure 1: Neurolamella depleted locust brain (*Schistocerca Gregaria*) ready for compound exposure and main brain regions in the locust brain



EntomoPharm

Ex vivo P-gp inhibition model

There is a strong evolutionary conservation between the insect blood brain barrier (BBB) and the mammalian BBB. This includes functional chemoprotective parallels between the human MDR1/P-gp transporter system and the insect Mdr65 transporter system [1]. EntomoPharm has developed an experimental *ex vivo* model based on the locust brain (Figure 1) for analyzing physiological properties of the BBB in the intact brain of locusts (*Schistocerca Gregaria*). The model can be used to screen small-molecule compounds for permeability and to identify P-glycoprotein (P-gp) interactions of drug leads [2]. The model can replace standard *in vitro* screen models in the early drug development screen cascade and supplement vertebrate models of BBB permeability.

In the **ex vivo P-gp Locust BBB model** the influence of P-gp inhibition on compound permeability is studied at constant test compound exposure of 1-10 μM and is independent of degrading enzymes, elimination and plasma protein binding. Data quality is high and the study outcome is always judged towards the response of an internal positive control.

Key model advantages:

1. The locust blood brain barrier is the only *ex vivo* model of BBB permeability that is based on an intact natural biological brain barrier that retains its biological integrity and control functions during the test procedure similar to vertebrate *in vivo* BBB models.
2. The **ex vivo P-gp Locust BBB permeability model** can be used to identify P-gp substrates and to rank BBB permeability in both single and multiple dosing regimens.
3. P-gp identification by pharmacological inhibition of P-gp in the **ex vivo P-gp BBB permeability model** avoids the complexities of multiple transporters by focusing specifically on effect of P-gp efflux inhibition.
4. The **P-gp Locust BBB permeability model** permits test on ready made stock solutions.

Locust *ex vivo* P-gp inhibition model

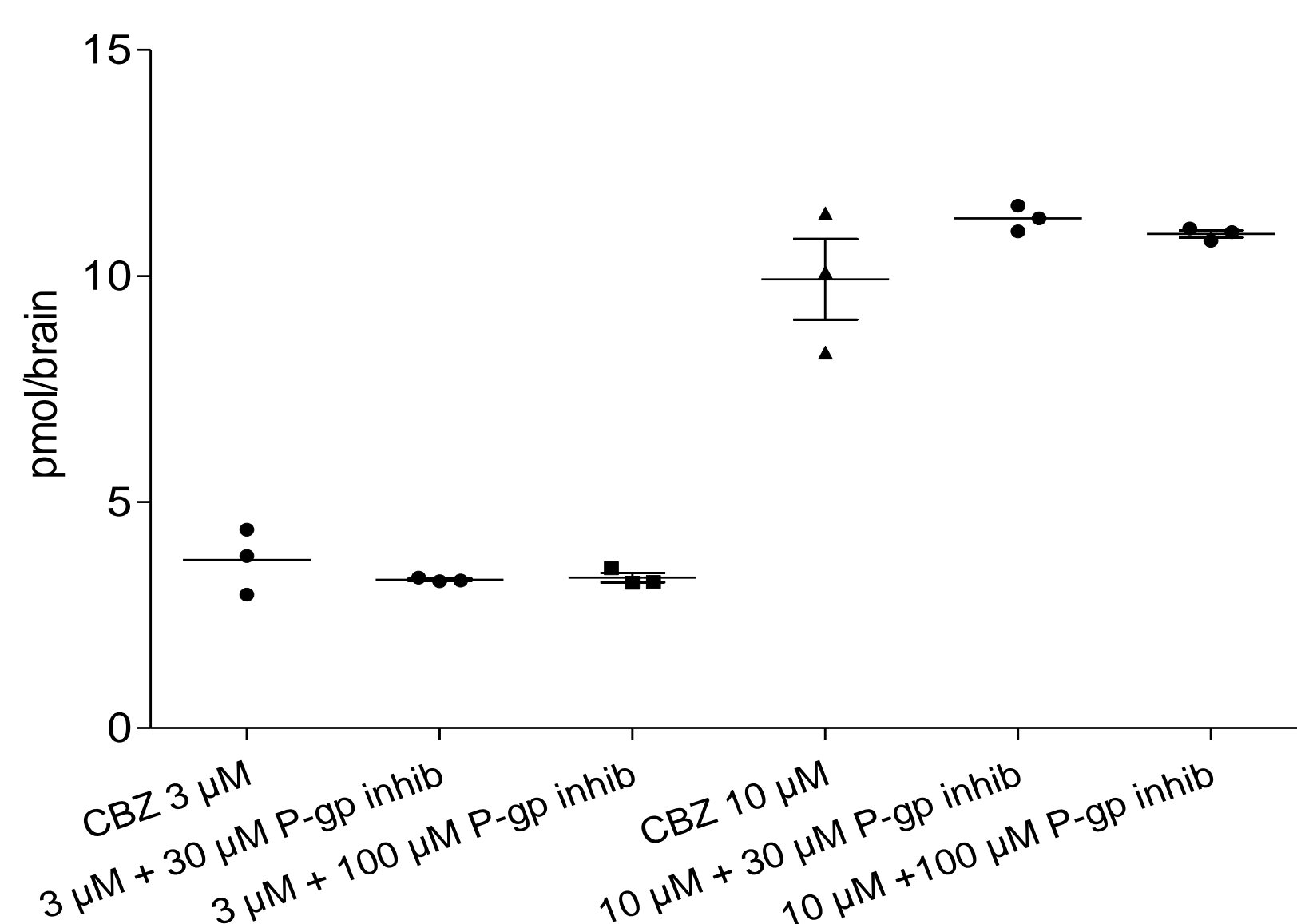


Figure 2: Locust brains exposed 5 min to carbamazepine (CBZ) at 3 μM and 10 μM with or without verapamil induced P-gp inhibition at 30 and 100 μM in the locust *ex vivo* P-gp inhibition model. * $P < 0.05$, ** $P < 0.005$ in an one way ANOVA, Newman-Keuls multiple comparison test ($n=3$)

Locust *ex vivo* P-gp inhibition model

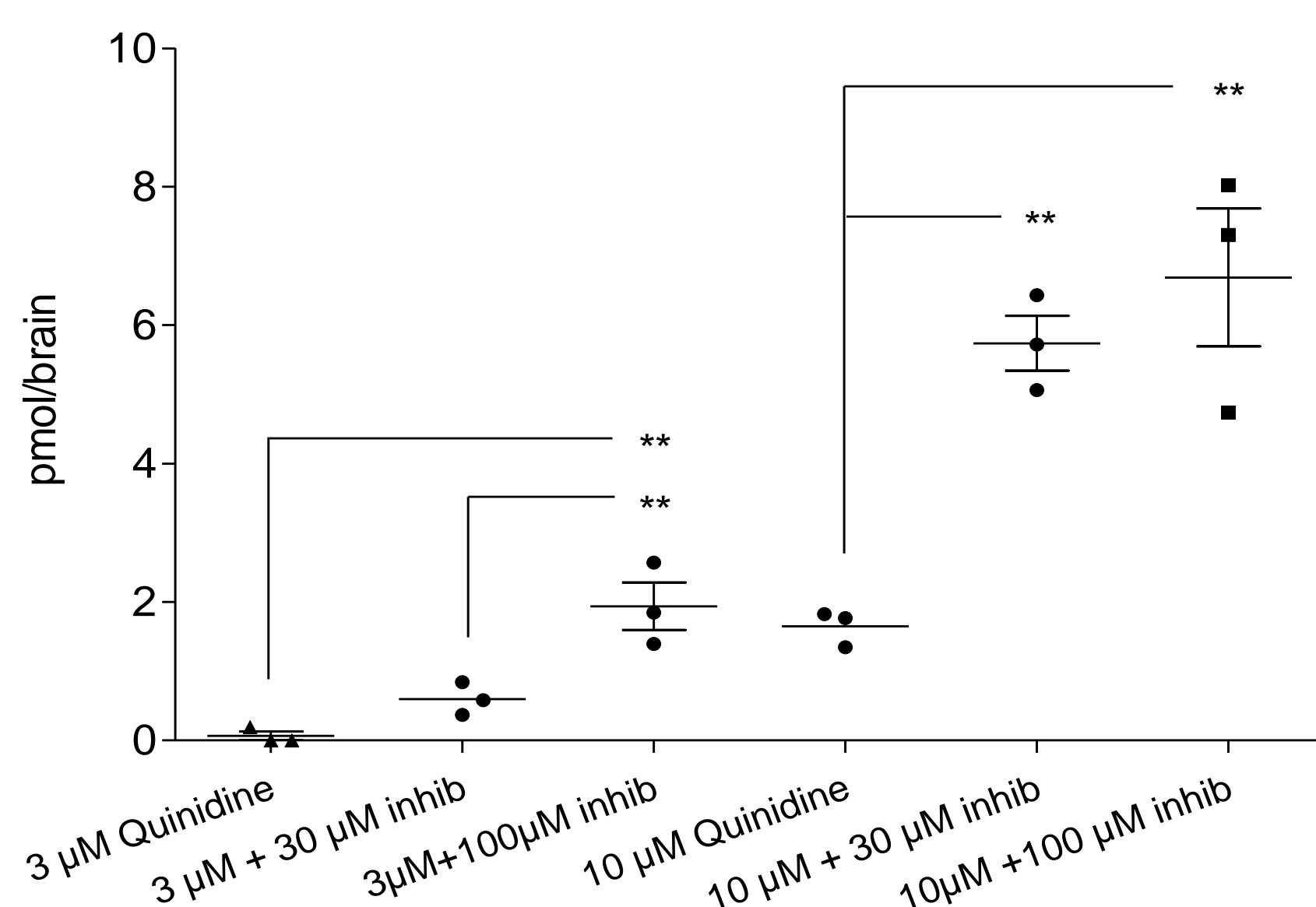


Figure 3: Locust brains exposed to the P-gp substrate quinidine at 3 μM and 10 μM with or without verapamil induced P-gp inhibition at 30 and 100 μM in the locust *ex vivo* P-gp inhibition model. * $P < 0.05$, ** $P < 0.005$ in an one way ANOVA, Newman-Keuls: multiple comparison test ($n=3$)

Figures 2 and 3 discussion

Co-administration of carbamazepine with the human P-gp inhibitor verapamil at 30 and 100 μM does not alter the BBB permeability of carbamazepine in either of the two doses 3 or 10 μM (figure 2) indicating that carbamazepine is not a substrate for the insect MDR1 analogue, Mdr65. This correlate well to verapamil induced P-gp inhibition results obtained from *mdr1a/1b(-/-)* knockout mice and Caco-2 (3)

Co-administration of the P-gp substrate/inhibitor quinidine with the P-gp inhibitor verapamil at 30 and 100 μM lead to increased brain uptake of quinidine in the two doses 3 μM and 10 μM (figure 3). Whereas BBB permeability of quinidine at 3 μM in co-administration with Verapamil at 30 μM is significantly less than in co-administration with verapamil at 100 μM indicating some residual P-gp efflux effect in the *ex vivo* P-gp inhibition model at this dose level.

About EntomoPharm

EntomoPharm was established in 2009 by multiyear experienced industrial professionals. The EntomoPharm vision is to become the global leader in cutting-edge insect models, delivering high-quality, high throughput and robust *in vivo* data in a timely and cost-efficient manner. EntomoPharm's insect platform is designed to enable strong R&D decision making, thereby reducing the present-day 50-60% late stage ADMET failures. EntomoPharm continually develop and optimize their pre-clinical screen models in insects to provide customers with cost-efficient, quality rich data to fit the needs of pre-clinical compound optimization programs.

1. Mayer F, Mayer N, Chinn L, Pinsonneault RL, Kroetz D, Bainton RJ: Evolutionary Conservation of Vertebrate Blood-Brain Barrier Chemoprotective Mechanisms in Drosophila. The Journal of Neuroscience, 2009 March 29(11), 3538 -50.
2. Nielsen PA, Andersson O, Hansen SH, Simonsen KB, Andersson G.: Models for predicting blood-brain barrier permeation. Drug Discovery Today, 2011 Jun, 16 (11-12), 472-5.
3. Owen A, Pirmohamed M, Tetley JN, Morgan P, Chadwick D, Park BK: Carbamazepine is not a substrate for P-glycoprotein. Br J Clin Pharmacol. 2001 April; 51(4): 345-349.

