Experiment 9
Transport Assay
Kinetics of GABA Uptake by the Brain GABA Transporter

This experiment examines the nature of γ-aminobutyric acid (GABA) uptake by one isoform of the brain GABA transporter (GAT3). The experiment involves expressing GAT3 in eggs harvested from female African clawed frogs (*Xenopus laevis*). This is accomplished by injecting the complementary RNA (cRNA) coding for GAT3 into the cytoplasm of oocytes. The cRNA in the cytoplasm is efficiently translated, the nascent protein folds properly in the membrane of the endoplasmic reticulum, the protein is packaged in transport vesicle, and finally targeted to the plasma membrane. Once in the plasma membrane the function of this protein can be examined by using a variety of methods. In our experiment we will perform a **transport assay**.

You will carry out the experiments using the Java applet **tassay**. There is a link to the applet next to this writeup. In the experiment, two cells are placed in a vial and are incubated for 30 minutes. One cell has the GAT3 transporter, and the other cell does not. The cell without the transporter will be called the "control cell". After the 30 minute incubation period, each cell can be placed in a liquid scintillation detector and the counts recorded. The applet also lets the user place 10 μl of the vial solution into the liquid scintillation detector.

The incubation solution in the vial contains a fixed amount of "hot" GABA and come "cold" GABA added. The applet allows the user to vary the amount of "cold" GABA added to the incubation solution. When the user clicks the "record" button, the applet displays the counts/min in the three channels: Channel A (0-10), Channel B (11-2000), and Channel C (0-2000). Note: there is a chance that quenching can occur.

Summary of Applet Functions

1. The slide bar allows the user to vary the amount of "cold" GABA added to the incubation solution.

2. The pull-down menu lets the user choose what will be placed in the liquid scintillation detector vial for counting. The choices are: 10 μl of incubation solution, the control cell (no GABA transporter), and the GAT3-expressing cell.

3. The record button displays the counts/min in the three channels of the liquid scintillation detector. Note: there is a chance that quenching can occur.

4. **All incubation solutions contain the same fixed amount of "hot" GABA**.

The Stock Solution:

You obtained the stock solution from Amersham Pharmicia Biotech, a commercial supplier of radioisotopes. You have purchased 250 μL of [^3H]-GABA. The certificate accompanying the isotope indicates that the **specific activity** of the isotope is 89.0 Ci/mmol. It also indicates that the **radioactive concentration** is 1.0 mCi/ml. The certificate notes that the calibration was done on January 15, 2002.

Writeup for the Transport Assay Experiment

1. (1 point) Based on the information of the stock solution, determine how many ^3H nuclei are on each GABA molecule.

2. (1 point) Based on the information of the stock solution, determine the [^3H]-GABA concentration in the stock sample purchased. Express your answer in mM (milli-molar).

3. (1 point) Determine the activity of [^3H]-GABA in the stock solution today. Express your answer in units of DPM/μL.

4. (1 point) The incubation solution (without any "cold" GABA) is a 5 mL solution to which 10 μL of stock [^3H]-GABA has been added. Use the applet to determine the counting efficiency of the detector for ^3H.

5. (3 points) Use the applet to run appropriate experiments to determine the transport rate of GABA into the control and the GAT3-expressing cells for different concentrations of GABA in the incubation solution. Make up a data table of the transport rate in units of pmol/min in terms of the GABA concentration.

6. (1 point) Plot the transport rates as a function of the GABA concentrations. Do this for both the control and the GAT3-expressing cells.

7. (3 points) Subtract the transport rates for the control cells from those of the GAT3-expressing cells, and fit the data to the Michaelis-Menten equation:

$$V = \frac{V_{max}[GABA]}{K_m + [GABA]} \tag{1}$$

where V is the transport rate (pmol/min), $[GABA]$ is the gaba concentration in the incubation solution, V_{max} is the maximum transport rate, and K_m is the GABA concentration that gives half the maximum transport rate.

8. (1 point) What values to you obtain from your fit for V_{max} and K_m?