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A Self-Study Tutorial using the Allen Brain Explorer and Brain Atlas to Teach Concepts of Mammalian Neuroanatomy and Brain Function

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The Allen Brain Atlas is a repository of neuroanatomical data concerning the mouse brain. The core of the database is a Nissl-stained reference atlas of the brain accompanied by *in situ* hybridization data for essentially the entire mouse genome. This database is freely available at the Allen Institute for Brain Science website, as is an innovative tool to explore the database, the Brain Explorer. This tool is downloaded and installed on your own computer. I have developed a self-study tutorial, "Explorations with the Allen Brain Explorer", which uses the Brain Explorer and the Brain Atlas to teach fundamentals of mammalian neuroanatomy and brain function. In this tutorial background information and step-by-step exercises on the use of the Brain Explorer are given using PowerPoint as a platform. To do the tutorial both the

PowerPoint and the Brain Explorer are opened on the computer and the students switch from one program to the other as they go, in a step-wise fashion, through the various exercises. There are two main groups of exercises, titled "The Basics" and "Explorations", with both groups accessed from a PowerPoint "Start Menu" by clicking on dynamic links to the appropriate exercises. Most exercises have a number of dynamic links to PowerPoint slides where background information for the exercises is given or the neuroanatomical data collected from the Brain Atlas is discussed.

Key words: Allen Brain Atlas; Brain Explorer; mouse brain neuroanatomy; olfactory system; limbic system; hypothalamus; hippocampus; reward center; in situ hybridization; gene expression

The Allen Brain Atlas (ABA), a project within the Allen Institute for Brain Science, is a mouse brain atlas that has been on-line since September 2006; it is publicly accessible at <http://www.brain-map.org>. The project combines databases generated through high-throughput *in situ* hybridization procedures with neuroanatomical data, primarily a database of Nissl-stained mouse brain sections. This latter database is referred to as the reference atlas. By combining *in situ* data with the reference atlas it is possible to create gene expression maps for the mouse brain (e.g. Lein et al., 2007). *In situ* data for most of the mouse genome are now in the databases.

To help navigate through the databases the ABA includes an exploration tool called the "Brain Explorer" (also available at <http://www.brain-map.org>). This is a desktop software application for viewing the Brain Atlas gene expression data in the framework of the reference atlas (Lau et al., 2008). The software constructs a 3-dimensional (3D) image of the mouse brain and allows the user to select coordinates within the model to gain access to the corresponding Nissl stained section from the ABA database (see Fig. 1); this same procedure also allows access to *in situ* data for the same selected brain region. The ABA and the Brain Explorer are used primarily as research tools (e.g. D'Souza et al., 2008; Mercer et al., 2008; Olszewski et al., 2008).

The Brain Explorer has features which makes it valuable as a teaching tool. It not only displays the mouse brain in 3D but also allows the user to turn-on and off the display of color-coded space-filled brain structures from which the virtual brain is constructed. These space-filled

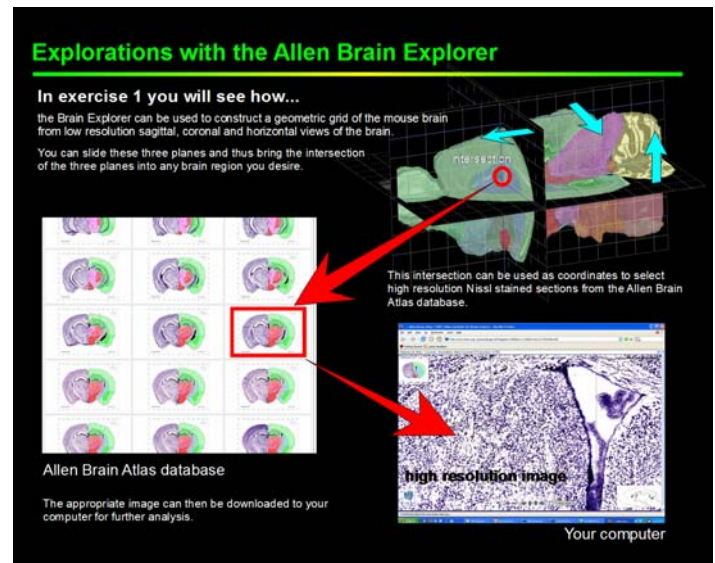


Figure 1. A screen shot from the "Introduction" to the tutorial. The introduction PowerPoint gives an overview of the subject matter of each exercise in the tutorial (shown above is an excerpt from the overview of Exercise 1).

structures can be displayed in a transparent mode making it possible to observe underlying regions within the model brain. Such features have tremendous educational value in allowing the user to visualize and construct the brain, structure by structure. These structures can be twisted and turned in space and the users can zoom in and out of the structures in their explorations of the brain. In addition

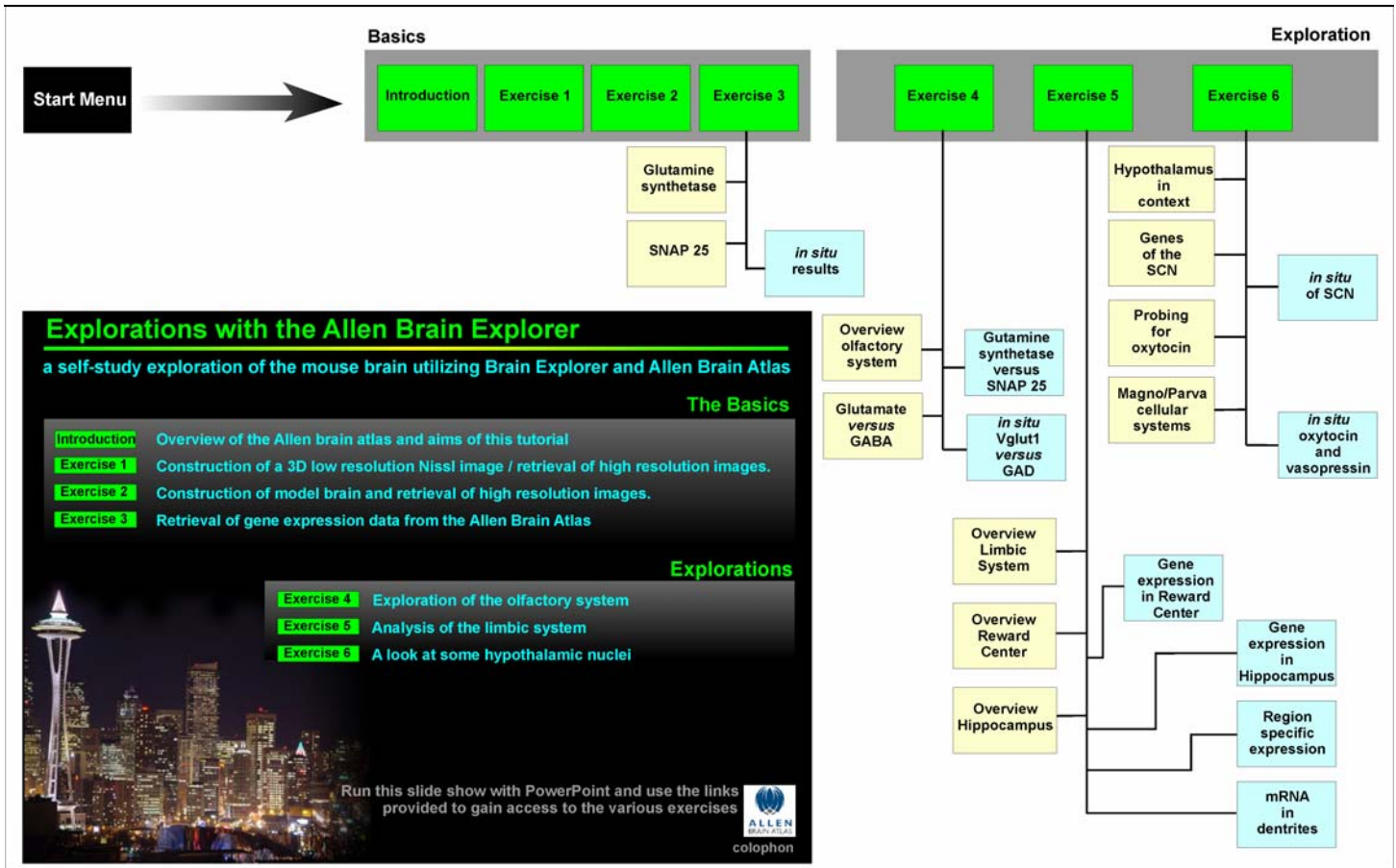


Figure 2. A flow-chart overview of the various PowerPoint documents that make up the tutorial; *Insert lower left:* a screen shot of the Start Menu. The Start Menu dynamically links to the “introduction” PowerPoint and to the PowerPoint slides of the six exercises. The first three exercises, titled “The Basics” give instructions on the use the Brain Explorer to construct models of the brain and how to gain access to high resolution data in the ABA; the last three exercises, titled “Explorations,” guide students in the use of the Brain Explorer to explore various aspects of brain structure and function. Most of the exercises possess links to PowerPoint presentations giving either additional background information (those shown in yellow) or a summation /discussion of the major finding in the exercise (shown in blue).

to these didactic features, one can superimpose gene expression information (in the form of color coded spheres) into the 3D models, making the Brain Explorer a remarkable tool for education in the neurosciences.

The tutorial “Explorations with the Allen Brain Explorer” has been developed with the idea of using the features of the Brain Explorer, outlined above, to give students an understanding of mammalian brain structure and function. It is targeted to undergraduate level biology (or biomedical) students. In its current form it has been designed to accompany an introductory neurobiology course in which it functions largely as a “self-study” program.

HOW THE TUTORIAL OPERATES

There are two key elements to the tutorial, namely a collection of “linked” PowerPoint presentations and the Brain Explorer itself (downloaded and installed on your computer). An overview of the PowerPoint documents that comprise the tutorial is given in Fig. 2. To run the tutorial both PowerPoint and the Brain Explorer are open. One simply follows the instructions given in the PowerPoint

presentations to operate the Brain Explorer. During the tutorial the students constantly switch back and forth between the PowerPoint slides and Brain Explorer as they go step-by-step through the instructions laid down in the exercises of the tutorial. While running the PowerPoint presentations the Windows taskbar can be displayed and used to go into the Brain Explorer, seamlessly, without interrupting the running of the PowerPoint.

OVERVIEW OF THE EXERCISES

To give an impression of the contents and aims of the tutorial a brief overview of the introduction and each exercise is given below.

Introduction: Overview of Allen Brain Atlas and aims of this tutorial

After an introduction to the ABA this presentation gives an account of the Nissl staining method which was used to construct the reference atlas. The Brain Explorer is then introduced and its function in accessing the databases of the ABA is considered. This is followed by a brief overview

of what will be accomplished in each of the exercises of the tutorial (e.g. Fig. 1). Finally, instructions are given on how to run the tutorial.

Exercise 1: Construction of 3D low resolution Nissl image and retrieval of high resolution images.

This exercise shows how to construct a 3D low resolution Nissl atlas of the mouse brain and then, using this atlas as reference, how to access full resolution images of selected brain regions. Sagittal and coronal views are explained, as is the use of the Bregma as reference point in brain mapping. The uses of many of the tools of the Brain Explorer are introduced in this exercise.

Exercise 2: Construction of model brain and retrieval of high resolution images.

A 3D model of the brain is created, piece by piece. The 3D model and the structures within it are then used to guide the user in accessing appropriate Nissl stained sections in the ABA reference atlas. In this exercise students acquire an insight into the 3D structure of the brain and the anatomical relationship between different brain regions (see Fig. 3).

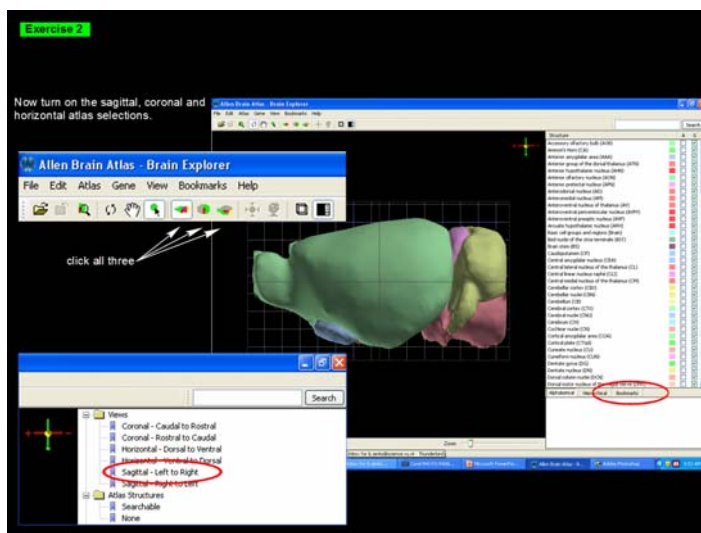


Figure 3: Screen shot from Exercise 2 giving instructions on how to manipulate the orientation and appearance of the brain model constructed with the Brain Explorer.

Exercise 3: Retrieval of gene expression data from the Allen Brain Atlas.

The Brain Explorer is used to access the ABA *in situ* gene expression databases. In this exercise various ways of displaying gene expression in the low resolution 3D mode are demonstrated (e.g. Fig. 4). These demonstrations use the expression of glutamine synthetase as a glial cell marker and expression of SNAP-25 as a neuronal cell marker. The exercise includes links to PowerPoint presentations explaining why these genes are good markers for the above cell-types. A demonstration of how to access high resolution *in situ* data for these same genes in a selected brain region is included in this exercise, as is a link to a presentation summarizing the expression of the two marker genes.

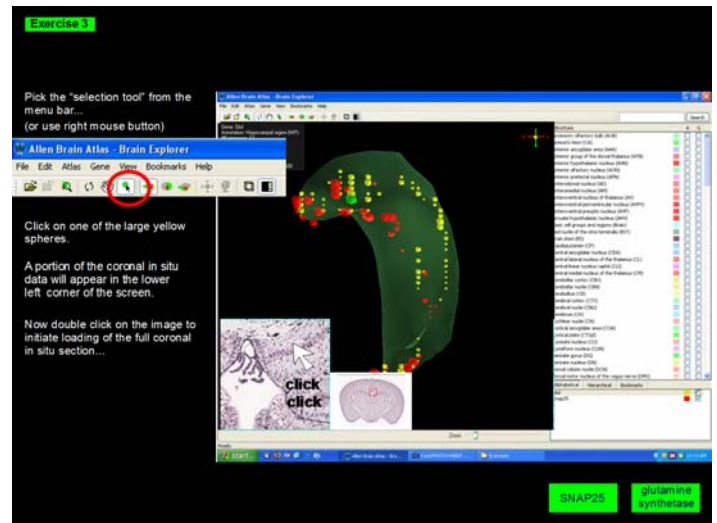


Figure 4: Screen shot from Exercise 3 showing low resolution display of the expression of two (color coded) genes in the hippocampus. Size of colored spheres give an indication of the level of gene expression.

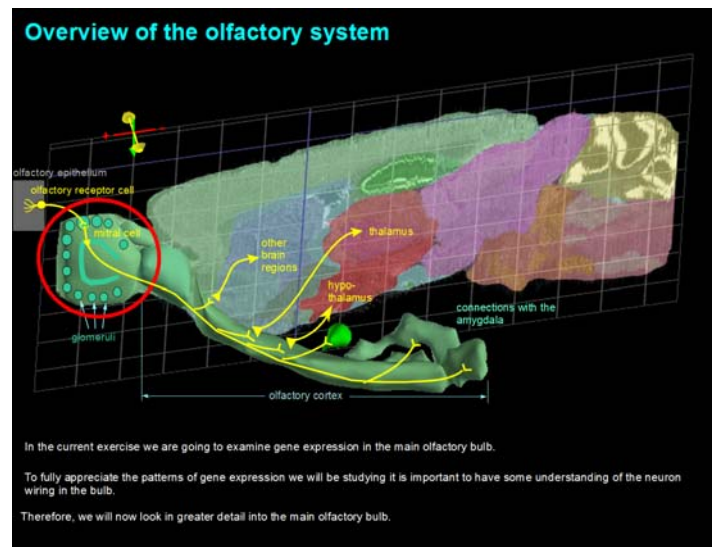


Figure 5: Screen shot from the overview of the olfactory system in Exercise 5. This prepares the user for an in depth analysis of gene expression in the olfactory bulb.

Exercise 4: Explorations of the olfactory system.

This is the first exercise of the "Explorations" phase of the tutorial. It starts by giving a link to a PowerPoint overview of the olfactory system (Fig. 5). A 3D model of the olfactory system is then constructed. The exercise then focuses on the olfactory bulb for an in depth examination of gene expression. First to be examined are SNAP-25 and Glutamine synthetase as markers for neuronal *versus* glial cells respectively. The expression of vesicular glutamate transporter (vGlut1) *versus* glutamic acid decarboxylase (GAD) are then examined. The expression pattern of these two genes give an impression of the distribution of excitatory *versus* inhibitory neurons, respectively. A presentation gives background information on why these two genes are good markers for such neurons. Finally, a

link to a summarizing PowerPoint presentation on the pattern of expression of these marker genes in the olfactory bulb is given.

Exercise 5: Analysis of the limbic system

This exercise starts with a link to a PowerPoint giving an overview of the limbic system and then guides the user in constructing a model of the limbic system (Fig. 6). It then goes on to consider the reward system, where expression of tyrosine hydroxylase (TH, the rate limiting enzyme for production of the neurotransmitter dopamine) and dopamine D1 and D2 receptors are examined. This analysis shows high expression of TH in neurons of the ventral tegmental area (VTA), the source of dopaminergic neurons in the reward system; this same analysis shows high expression of dopamine receptors in the nucleus accumbens, which is one of the important target areas for VTA neurons (Fig.7).

Following the analysis of the reward center attention in this exercise shifts to the hippocampus, starting with a link giving background information on this brain region. In this exercise three aspects of gene expression in the hippocampus are then considered, namely (1) expression in neurons *versus* glial cells, (2) regional specific gene expression among neurons of the hippocampus (see Fig. 8), and (3) the presence of mRNA in dendrites of CA1 pyramidal neurons.

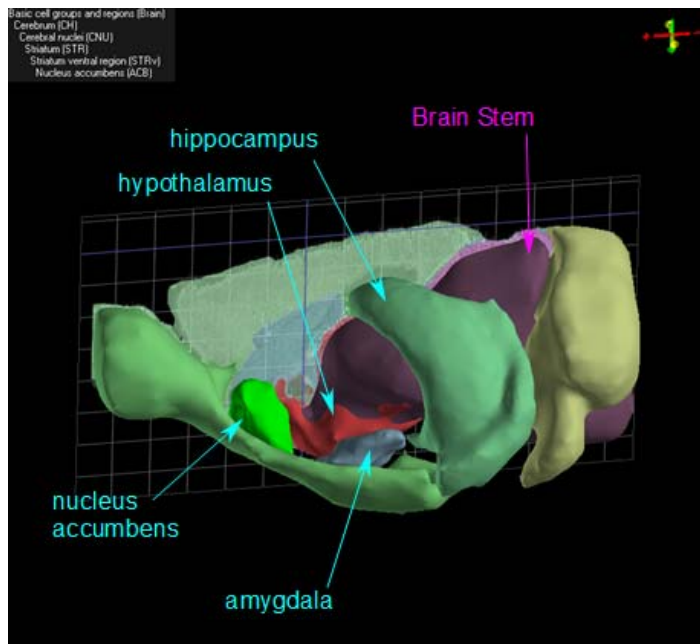


Figure 6: Model of part of the limbic system as constructed with The Brain Explorer in Exercise 5.

Exercise 6: A look at some hypothalamic nuclei

Following an introductory PowerPoint concerning the general function of the hypothalamus, this exercise first considers the suprachiasmatic nucleus (SCN), the site of the so-called “biological clock” responsible for driving circadian rhythms throughout the body. A link to a brief overview of the SCN and “clock genes” is provided and the

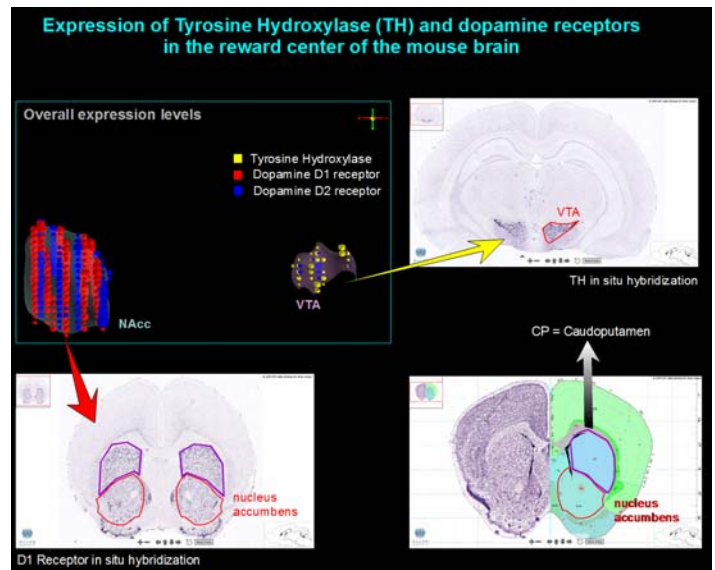


Figure 7: Analysis of gene expression concerning dopamine production (enzyme TH) and dopamine receptors (D1 and D2 receptors) in the reward center. This is a screen shot from a presentation summarizing the analysis of the reward system in Exercise 5.

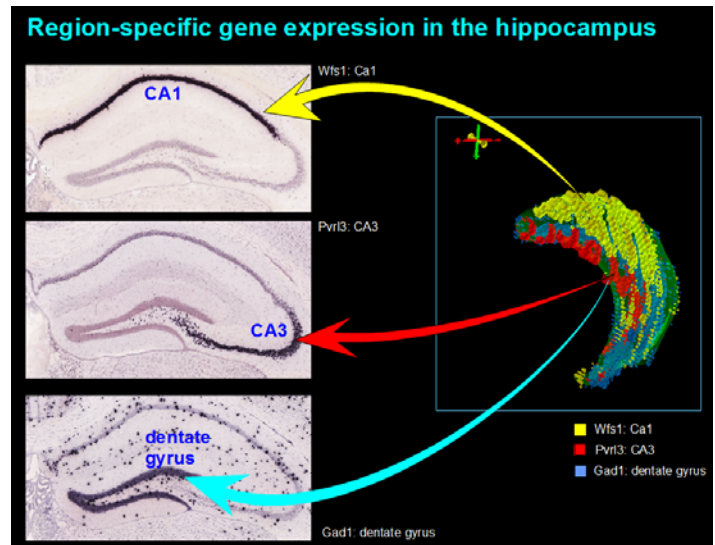


Figure 8: Regional specific gene expression in the hippocampus. This is a screen shot from one of the summarizing presentation in Exercise 5.

expression of some “clock genes” then examined. Attention in the exercise then shifts to two nuclei, the suproptic nucleus (SON) and paraventricular nucleus (PVN), which produce the neuropeptides oxytocin and vasopressin. To introduce oxytocin, a link to a PowerPoint is provided giving details on the production of the *in situ* probe for oxytocin (this presentation is intended to illustrate the general approach used to produce the probes for construction of the ABA). The expression of oxytocin and vasopressin in the SON and PVN is then examined and expression of the receptors for these neuropeptides also receives attention. To conclude this exercise the expression of corticotrophin releasing hormone (CRH) is

examined. In considering CRH, a background PowerPoint presentation gives an overview of the Magnocellular and Parvocellular systems of the hypothalamus. This presentation clarifies the position of CRH, vasopressin and oxytocin within these systems. Fig. 9 gives a screen shot of the final projection of this exercise in which the overall structure within the tutorials can be seen. The image of the Brain Explorer takes a central position with instructions or comments being aligned on the left of the screen and hyperlinks to PowerPoints associated with the exercise being placed in the upper and lower right of the screen.

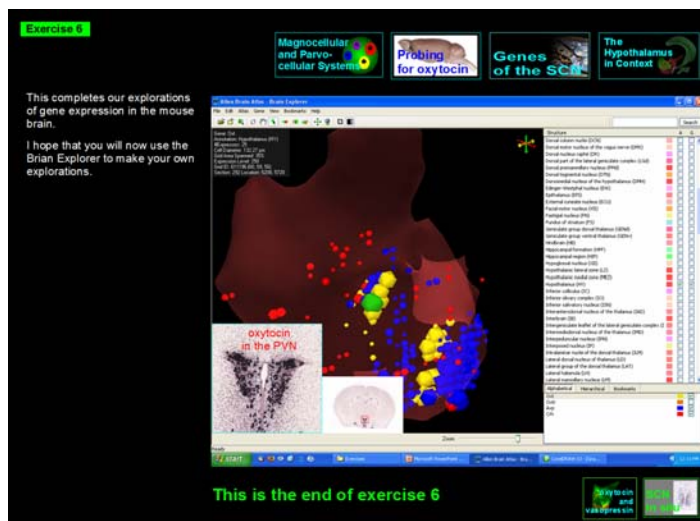


Figure 9: Final projection in Exercise 6. The general structure of the tutorial can be seen with links to background material inserted in the upper right corner of the screen and links to summarizing presentations of the material covered in the exercise placed in the lower right corner.

DISCUSSION

This tutorial has been designed to run as a self-study program within the context of an introductory undergraduate level neurobiology course. Its primary function is to give students insight into mammalian brain structure and function. Considerable background material is included within each exercise in the form of linked PowerPoint presentations (represented by the yellow boxes, Fig. 2) so that the tutorial can run independent of the lecture material. Ultimately most of the background material of the tutorial is covered in the lectures, so the tutorial backs up the lectures and *vice versa*; and therefore, the tutorial and lecture material become well integrated.

The use of PowerPoint as a platform for this tutorial has advantages over other perhaps more obvious choices such as a HTML-based platform or use of animation software such as Adobe Flash. The biggest advantage is the ease of programming. Almost everybody in an academic setting is familiar with PowerPoint and has some experience in constructing PowerPoint presentations. It follows that, with PowerPoint as platform, most instructors can very easily make alterations to the program to streamline it to their own particular needs. If one aspect of an exercise is not relevant to the current course, then it can be deleted from the PowerPoint. Likewise, new material can be added to

the existing PowerPoint documents to meet unique needs. Complete new exercises can easily be created and linked in the Main Menu to build upon the preexisting exercises.

There are several avenues for future development in the tutorial. In the current tutorial students are encouraged, at a number of points in the exploration phase, to conduct their own exploration, thus building upon explorations already initiated. A new development could be the inclusion of exercises asking students to conduct completely new explorations into brain regions or structures not yet explored. For example, they could be asked to study gene expression in the choroid plexus. Here they would soon discover that the best way to illuminate the plexus is to image expression of genes involved in pumping electrolytes across membranes. As an extension to this approach, they could be asked to make a report of their exploration, similar to the way the current tutorial gives summaries of each exploration. The students could even be asked to create a new module for the tutorial based on their explorations.

Another avenue for future development could be the organization of a platform (such as a website) for obtaining the tutorial, for exchanging information and ideas, and perhaps even developing and exchanging new modules for the tutorial. Instructors could select the most appropriate modules for their course, download them and link them to the Main Menu. In this way a new tutorial could quickly be created that would meet course requirements in a unique way. An MS Word step-by-step outline of the first three exercises can be found at <http://community.brain-map.org/confluence/display/EDU/Home>.

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