

7.22 Fall 2005

study questions for exam 2

Three Study Questions from MCP for Exam II 7.22 Nov 7, 2005

1) You are interested in establishing the beginning and end of the period in the cerebral cortex of the tasmanian devil (a fierce marsupial found in Tasmania Australia) when cerebral cortex neurons are first born and when neurogenesis is complete. You would also like to establish whether the pattern of cell migration and settling is the same in the tasmanian devil and in mammals such as the ferret, mouse and rhesus monkey. Consequently using bromodeoxyuridine you pulse label tasmanian devils in utero beginning on embryonic day 24, another litter is pulse-labeled at embryonic day 29 another set of newborns are pulse-labeled on the day of birth and a final set of animals are pulse labeled on postnatal day 4. You find the following pattern of bromodeoxyuridine labeled cells when the cortices of these devils are examined as young juveniles.

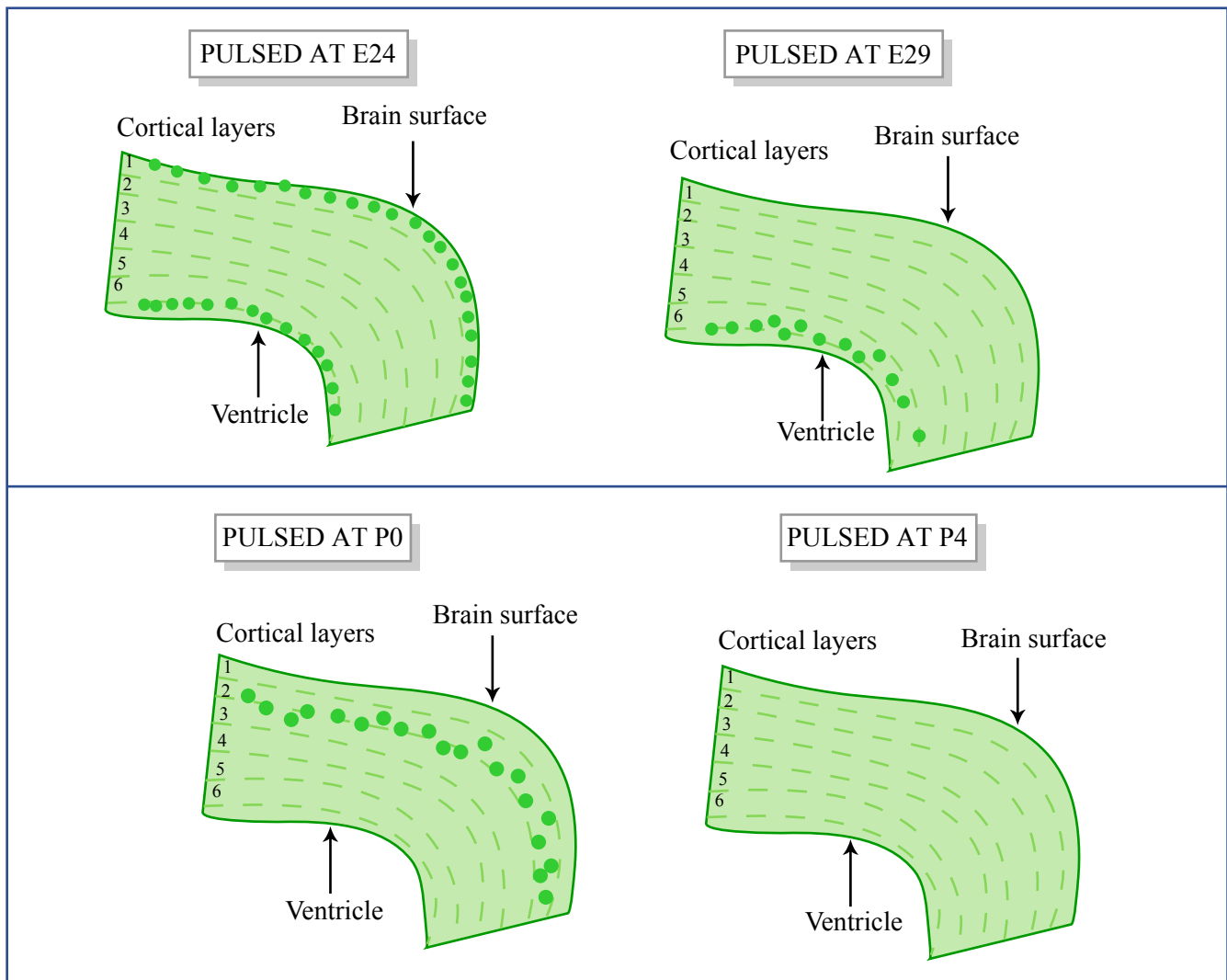


Figure by MIT OCW.

- When are cortical layer 6 cells born?
- When are cortical layer 2/3 cells born?

c) Which cells have the longest migration path?

d) You compare your pulse labeling pattern with that of ferrets pulsed at comparable stages of development. You find that young juvenile ferrets have no labeled cells above layer 2/3 or below layer 6 when pulsed at a stage comparable to E24 in the tasmanian devils. However you are surprised to find when you examine younger (P6) ferrets pulsed at the “E24” stage that they show the same bilayer pattern of expression as the tasmanian devil. Can you suggest an explanation for what is going on between the P6 and young juvenile stage in the ferret cerebral cortex that is not going on in the young tasmanian devils cortex?

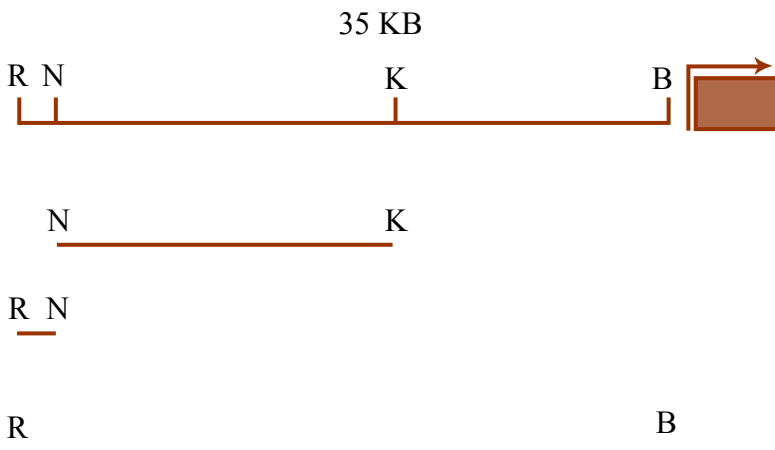
e) Suggest an experiment that would test your hypothesis.

f) Would you have detected the bilayer pattern of cell genesis after an E24 pulse of BrdU if you had instead used a retrovirus with a reporter gene to label a few progenitors at E24?

2) Exposure of *Xenopus* embryos to retinoic acid (RA) results in expansion of the *Xhox 6* gene into presumptive midbrain regions of the animal and a “posteriorization” of the embryos.

You obtain the upstream flanking region of the *Xhox 6* gene and use restriction enzymes R, N, K and B to produce the constructs illustrated below that you introduce into *Xenopus* neural tube stage embryos bilaterally using electroporation.

You next take the groups of these embryos transfected with these constructs and divide each construct group into 2 experimental groups: those treated with exogenous RA (w/RA) and those not treated with exogenous RA (w/o RA). You obtain the following data on LacZ expression in the midbrain and forebrain.



MB-r2		r3		r4-r8	
w/RA	w/o RA	w/RA	w/o RA	w/RA	w/o RA
-	-	+	+	+	+
+	-	+	+	+	+
+	-	+	-	+	+

a) What region of this 5' sequence appears to confer restriction of Xhox6 to r4-r8 in the 'normal' embryos (the embryos untreated with RA)? Explain your reasoning.

b) Where in this regulatory region is the retinoic acid responsive element (RARE)? Explain your reasoning.

3. A variety of reagents and approaches are frequently used by developmental biologists to understand the tissue interactions and molecular signaling pathways involved in particular aspects of development. Several of these approaches or reagents are listed below. To determine how well you understand "how we know what we know" in systems where the forward genetics used in fruit flies and worms is difficult (at best) find one example from your reading and lecture notes where this type of approach was used to demonstrate an important developmental principle. Give the example and the developmental principle illustrated.

a) Over-expression of a mRNA in the *Xenopus* zygote.

b) Cross-species injection of an orthologous gene from *Drosophila* into a developing vertebrate embryo.

c) Transgenic mouse carrying the entire regulatory region of *Hoxb1* in front of the reporter gene *LacZ*.

d) Knockout mouse for a known patterning gene.

e) An incapacitated retrovirus with a strong promoter driving *LacZ* or green fluorescent protein genes.

f) Exposure of a specified but undetermined tissue to different "inducing" tissues hypothesized to change the prospective fate of cells.