BIOL 430-Molecular Genetics of Development

Pregnancy-Stimulated Neurogenesis in the Adult Female Forebrain Mediated by Prolactin

Me

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Presenter: Michelle Wang

Seminar Outline

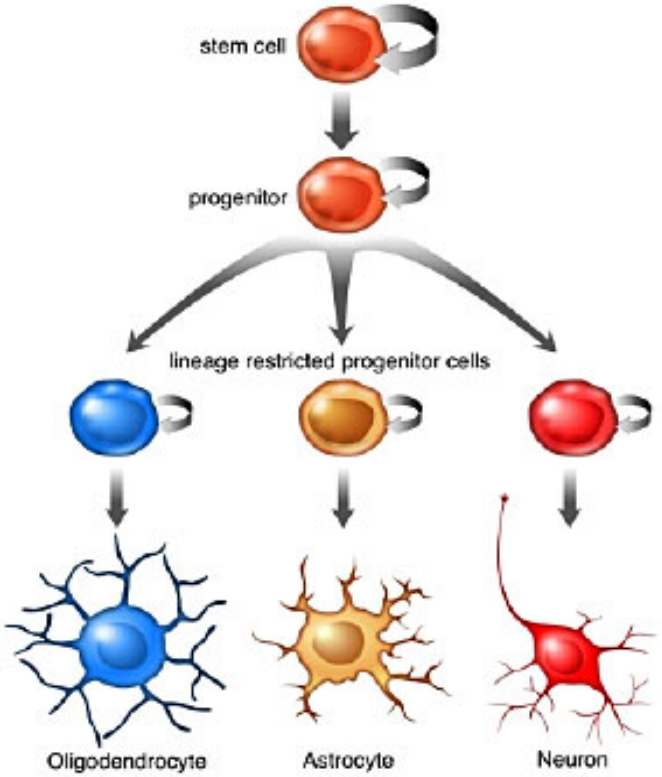
- Introduction
  - Neurogenesis
  - Discovery and comparative studies
  - Pregnancy hormones
- Methods and Results
- Strength and Limitations
- Progress in Neurobiology
  - Prolactin role in neurogenesis
  - Induction of neurogenesis
- Future Directions and Research
- Conclusion

Neurogenesis

- Neurogenesis is a process by which neurons are generated.
- Neurons are created in predominantly two areas of the brain.
  - Subventricular zone (SVZ)
    - new cells migrate to the olfactory bulb via the rostal migratory stream
  - Subgranular zone (SGZ)
    - part of the dentate gyrus of hippocampus

Neural stem cells (NSCs)

- Self-renewing, multipotent cell
- Can differentiate into three main lineage cell types of the nervous system
  - Neurons
  - Astrocytes
  - Oligodendrocytes



http://www.rienstraclinic.com/newsletter/2007/images/2007Jan_2neurogenesis.jpg

Pathway of neurogenesis in the SVZ

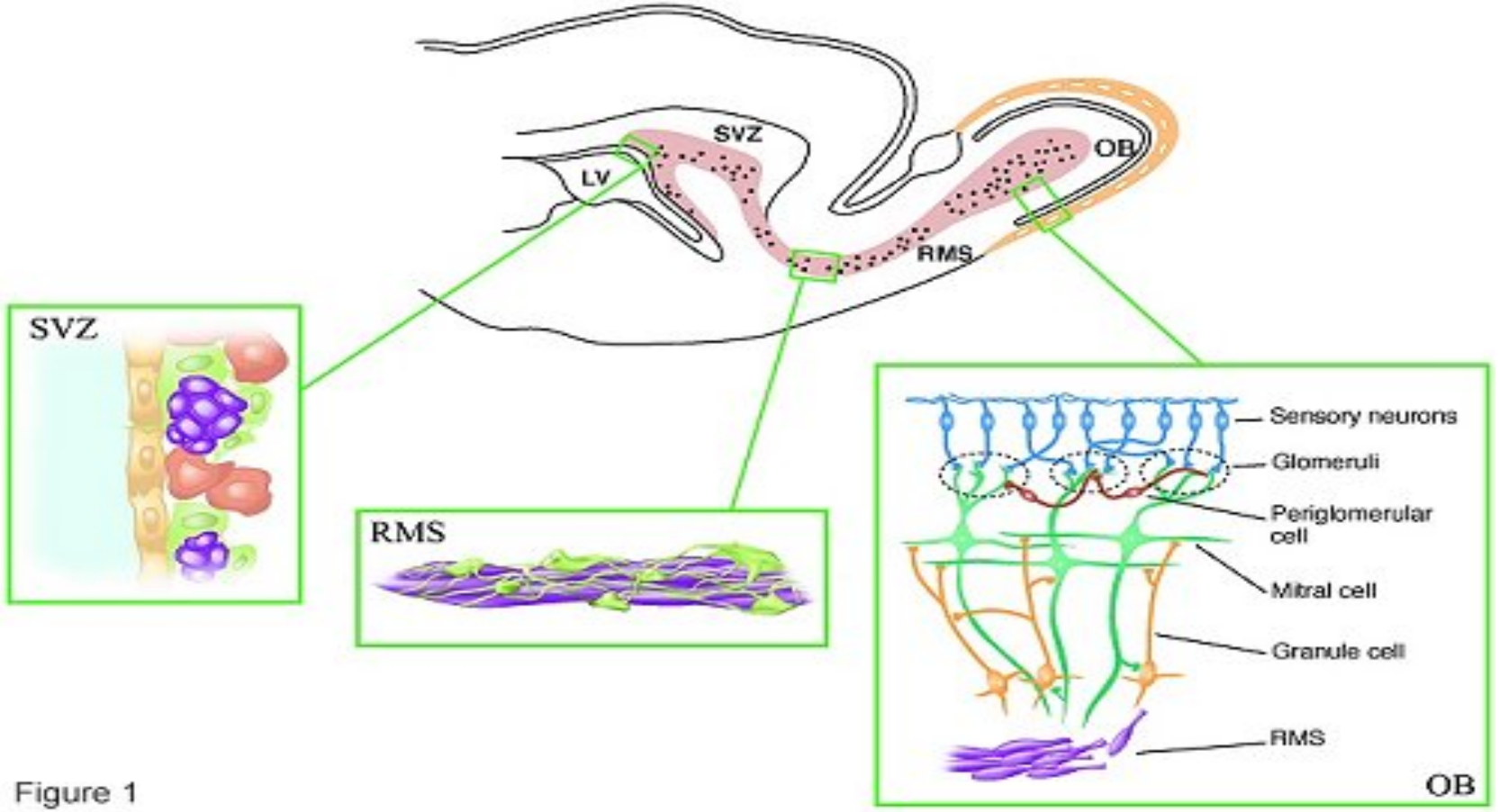


Figure 1

Purple: A cells (neuroblast)
Green: B cells (astrocytic features, divides slowly, ensheath migrating type A cells)
Red: C cells (derived from type B cells, rapidly dividing, gives rise to type A cells)
Type B → Type C → Type A

www.crulrg.ulaval.ca/.../Saghatelyan_Fig1.jpg

Pathway of neurogenesis in the SVZ

- Proliferating cells (precursor cells)
- Differentiation into an immature neurons
  - Neuroblasts (Neuronally committed)
- Migration of cells to olfactory bulb
- Within few days-grow and mature into fully functional neuron
  - Granule and periglomerular neurons

What affects neurogenesis?

- Corticosterone (stress) and antidepressants were found to affect cell proliferation in hippocampus
- Scheffler *et al.* (2005) study shows that:
  1) corticosterone decreases cell proliferation in SVZ
  2) antidepressant promotes SVZ cell proliferation
- Aging decreases cell proliferation

"no new neuron" dogma

1965-Altmas & Das

- First found newly born neurons
- Not able to locate them

1980s-Goldman

- Found neurons in canaries' high vocal center (HVC)
- HVC involved in song production

Nottebohm Team

- Discovered neurogenesis in hippocampus (spatial learning) in chickadees
- New neurons increased during song learning

Neurogenesis found in adult mammals

Weiss and Reynolds (1992)

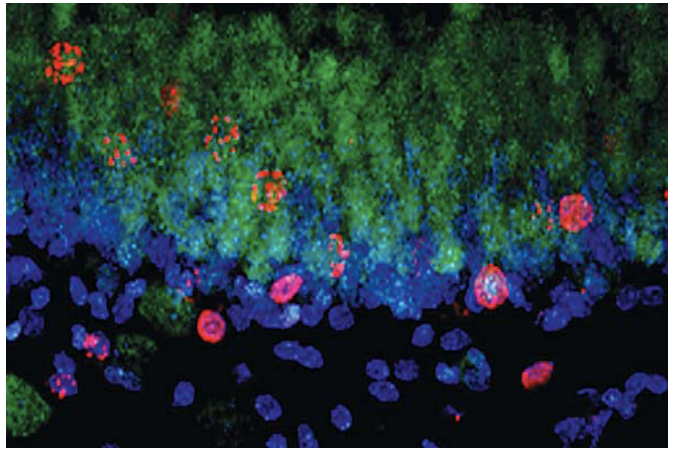
- Isolated NSCs from SVZ of mouse brains

Alvarez-Buylla and Lois (1994)

- Showed NSCs form neurons that travel to OB (brain area that receive sensory info from nose)

Gage *et al.* (1996)

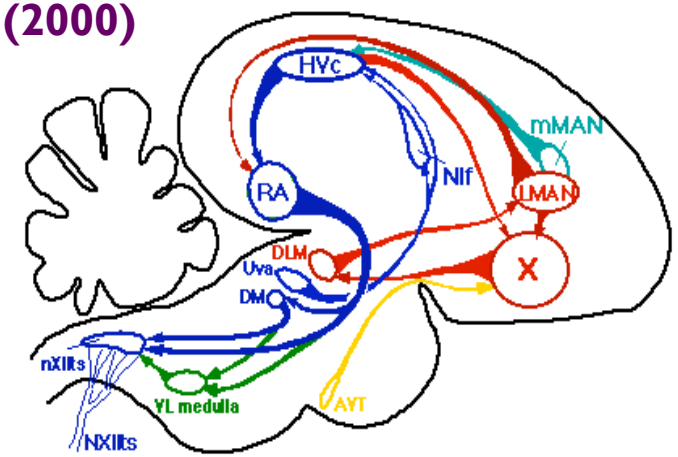
- Found new neurons in hippocampus (memory) of adult mice and humans



What do these new neurons do?

1. Song Production-Nottebohm *et al.* (2000)

- New neurons migrated to HVC for replacement
- Loss of neurons in HVC=song degraded
- Suggested neurogenesis vital in song production



http://people.eku.edu/ritchisong/RITCHISO/songbraincircuits.gif

2. Odor Recognition-Lledo *et al.* (2000)

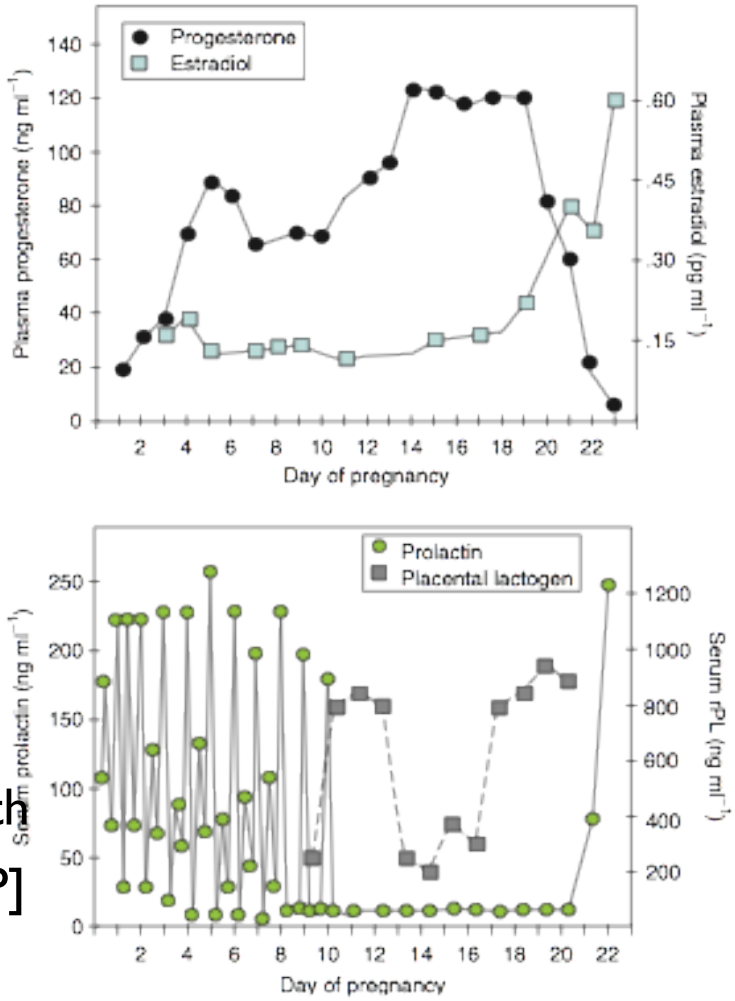
- New neurons join granule cells, which are activated by neurons that respond to odors
- Inhibit neighbouring cells=lateral inhibition
  - Sharpen neural activity evoked by an odor

Pregnancy is an adaptive state

- Pregnancy involves physiological changes in all major body systems.
- Pregnancy also induces unique maternal behaviours pre- and post-partum.
- Precise regulatory control of these processes is essential for long-term health of mother and children: regulation by Endocrine System

Neuroendocrine System: Circulating Hormones in Pregnancy

- Steroid Hormones: Estrogen (E2) and Progesterone (P)
- Complex Reg. Loop: Pituitary, Adrenal, Placenta, Ovaries
- Peptide Hormones: Prolactin (PRL) and Placental Lactogens (PL)
- Normally PRL is auto-regulated: Short Feedback Loop
- Early Pregnancy: PRL spikes twice daily
  - Controlled by E2 and P
- Late Pregnancy: PRL increases up until birth
  - Thought to be caused by decreasing [P]



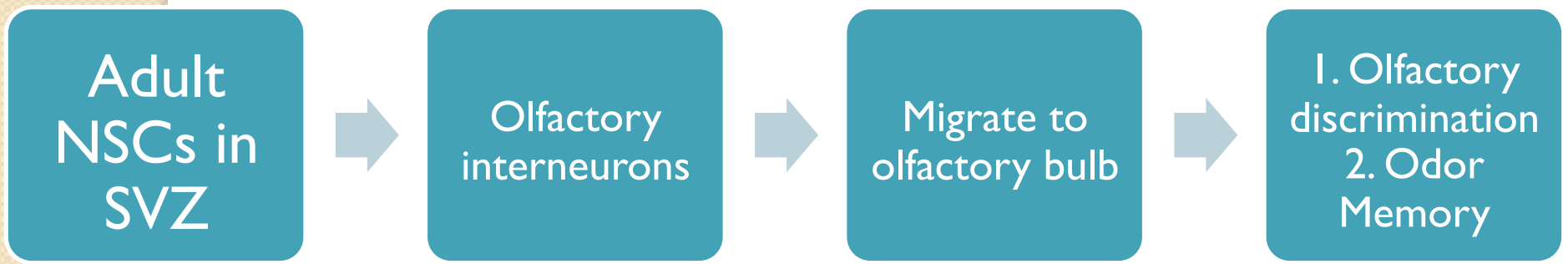
Pregnancy induces changes in behaviour

- What is maternal behaviour?
  - Parturitional: Placentophagia
  - Young-directed: retrieval, grooming, nest-building
  - Young-related: Aggression, ↑ Food consumption, ↓ anxiety
- Neuroendocrine control of maternal behaviour is well established
  - Estrogen, progesterone and prolactin
- Many maternal behaviours involve olfaction and new memories:
  - It is likely that the rate of neurogenesis at SVZ +/or DG may increase in pregnancy in order for the mother to show adaptive behaviours

Introduction: Pregnancy-stimulated neurogenesis

- Neurogenesis occurs in olfactory system of adult brain throughout life
- olfactory neuroblast proliferation and differentiation conserved in many species
  - Physiological regulation not understood
- Disruption of neuroblast to OB = deficits in olfactory discrimination

Overview:



Why is the study important?

Olfactory Discrimination is critical for:

- Offspring recognition
- Rearing young

Forebrain Neurogenesis may contribute to:

I. Mating
II. pregnancy

Purpose of Study:

Part I: Does pregnancy induce neurogenesis?

Part II: What physiological regulations of pregnancy contribute to the stimulation of neurogenesis?

Part I: Does pregnancy induce neurogenesis?

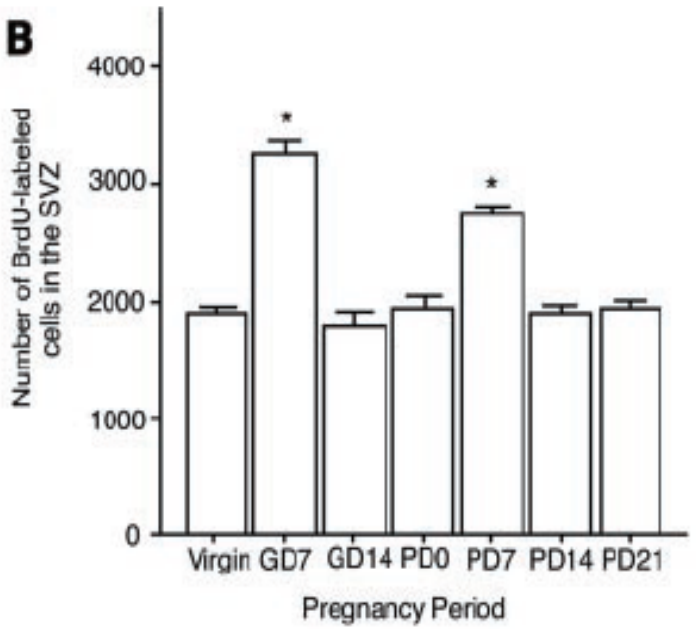
Study 1: Cell Proliferation in forebrain SVZ

Study 2: Pregnancy-induced neurogenesis in OB

Study 1: Are cells proliferating in forebrain SVZ in pregnant mice?

- Methods: Inject virgin and timed pregnant 6-8 weeks old CD1 mice with **bromodeoxyuridine (BrdU)**
  - Commonly used to detect proliferating cells in living tissue, marks DNA synthesis

- Results:
  - Increased proliferating of cells in forebrain SVZ at GD7(by 65%) and PD7(35%).
  - Returned to baseline at GD14, PD14, PD21.



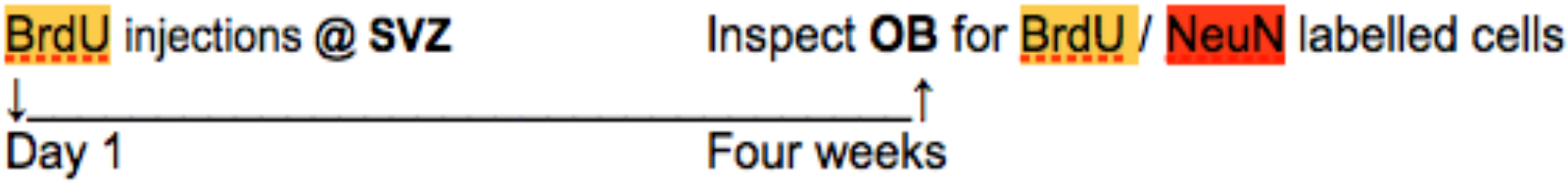
Cells are proliferating in forebrain SVZ in pregnant mice

- Two additional experiments were performed to confirmed the specific and selective nature of this increase

  1.) Ki67 (cellular marker for proliferation) increased by ~100% in GD7 forebrain SVZ

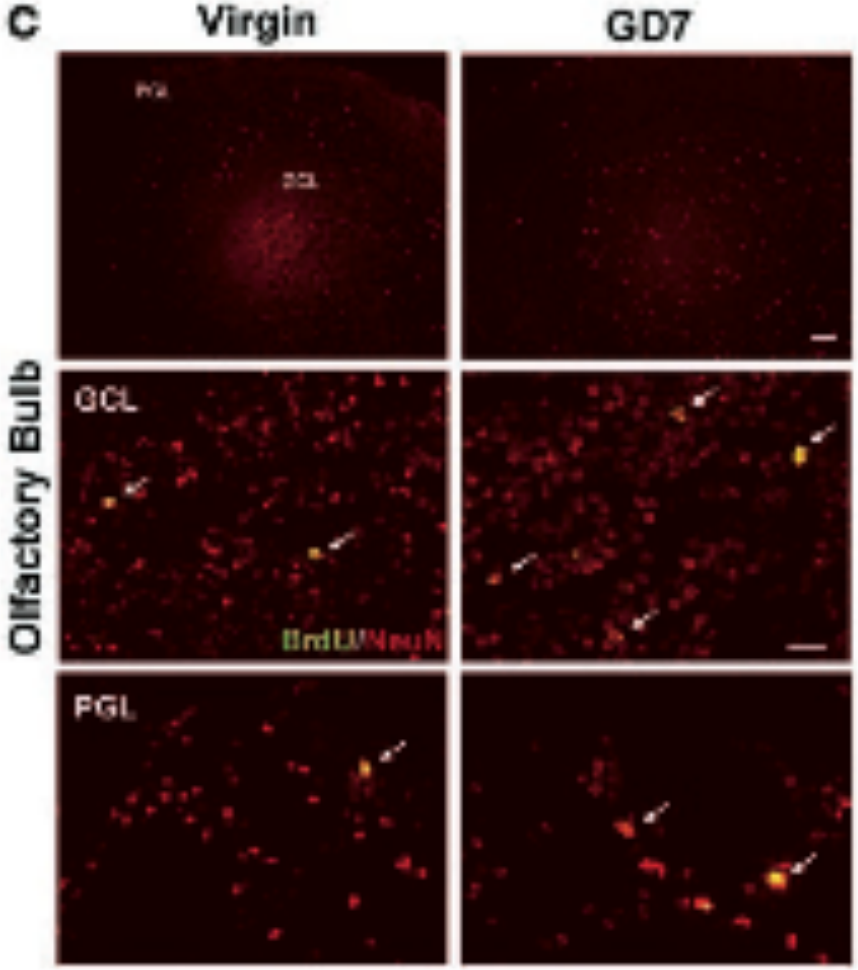
  2.) Number of BrdU-labeled cells in hippocampal dentate gyrus were not different between GD7 and virgin mice

Study 2: Does Pregnancy induce neurogenesis in the olfactory bulb?

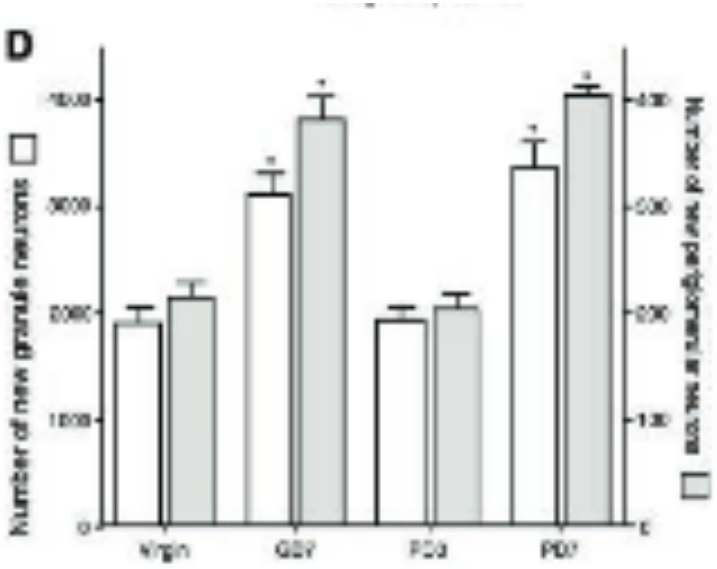


- Five BrdU injections to limit false negatives
- Four weeks gives time for migration, differentiation and apoptosis
- NeuN antibody staining specifies a neuronal phenotype
- Both the Granule Cell Layer and Periglomerular Cell Layer were examined

Proliferating Cells Do Migrate to OB and Differentiate into Neurons



[ ] Newborn Cells
[ ] Neuronal Cells



Conclusion: The increase in cell proliferation during pregnancy leads to a doubling of new-born interneurons in the olfactory bulb

Part II: What mediates neurogenesis during pregnancy?

Study 3: How does pregnancy modulate neurogenesis in forebrain SVZ?

Study 4: Does prolactin mediate neurogenesis?

Study 5: Where are prolactin receptors localized?

Study 6: What is the effect of PRL on NSC proliferation and differentiation?

Study 7: Is neurogenesis dependent on prolactin receptor?

Study 3: How does pregnancy modulate neurogenesis in forebrain SVZ?

a) Embryo Implantation
b) Maternal hormones

Study 3a: Distinguish roles of maternal hormones from other physiological signals during implantation

Method: mated virgin females with vasectomized males which resulted in pseudopregnancy

Results:

- Found more BrdU-labelled cells in forebrain SVZ in pseudopregnant females
- suggested that embryo implantation not necessary to stimulate neurogenesis and circulating maternal hormones sufficient to stimulate forebrain neurogenesis

Study 3b: Are maternal hormones, estrogen, progesterone or both the mechanism?

Method: Estrogen, progesterone or both injected into normal or ovariectomized female mice in brain or peripherally

Results: estrogen and/or progesterone failed to increase BrdU-immunoreactive cells in SVZ

Conclusive findings:

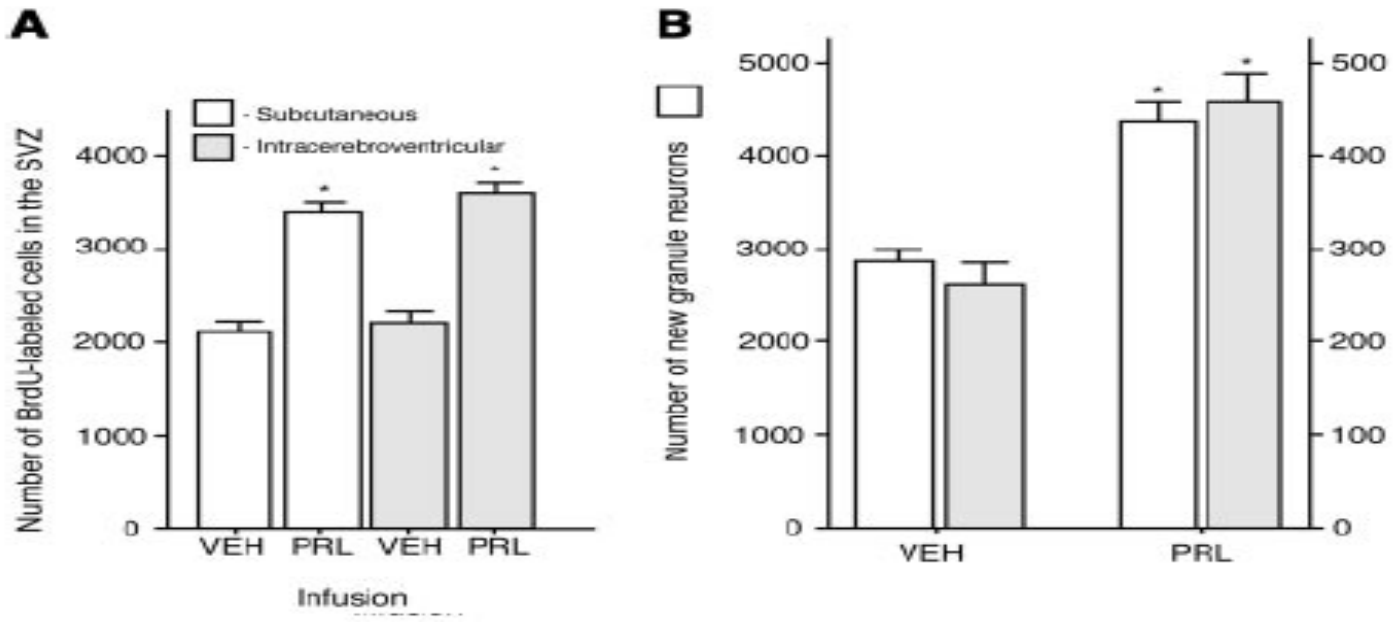
1. Embryo implantation does not stimulate neurogenesis and maternal hormones may account for increases in SVZ neurogenesis
2. Estrogen and/or progesterone did not mediate this

Study 4: Is prolactin responsible for this increase?

- Infused prolactin (PRL) into 6-8 weeks old ovariectomized mice either by
  - Subcutaneously (sc) or intracerebroventricularly(icv) for 6 days.

- Results
  - Increased BrdU-labeled cells in forebrain SVZ in both sc (53%) and icv (61%) routes
  - Numbers of new olfactory interneurons doubled after 4 weeks

Prolactin is responsible for this increase



- Increase of BrdU-labeled cells in SVZ after prolactin infusion
- Prolactin-induced mice increases in new interneurons in the olfactory bulb

Effects of Prolactin in males

- Prolactin and its receptors are also present in males
- Infused PRL or PRL-releasing peptide into the lateral ventricles for 6 days
- Results:
  - Increased proliferation in the forebrain SVZ in 6-8 weeks old male mice
  - Slightly less extent than that seen in age-matched females

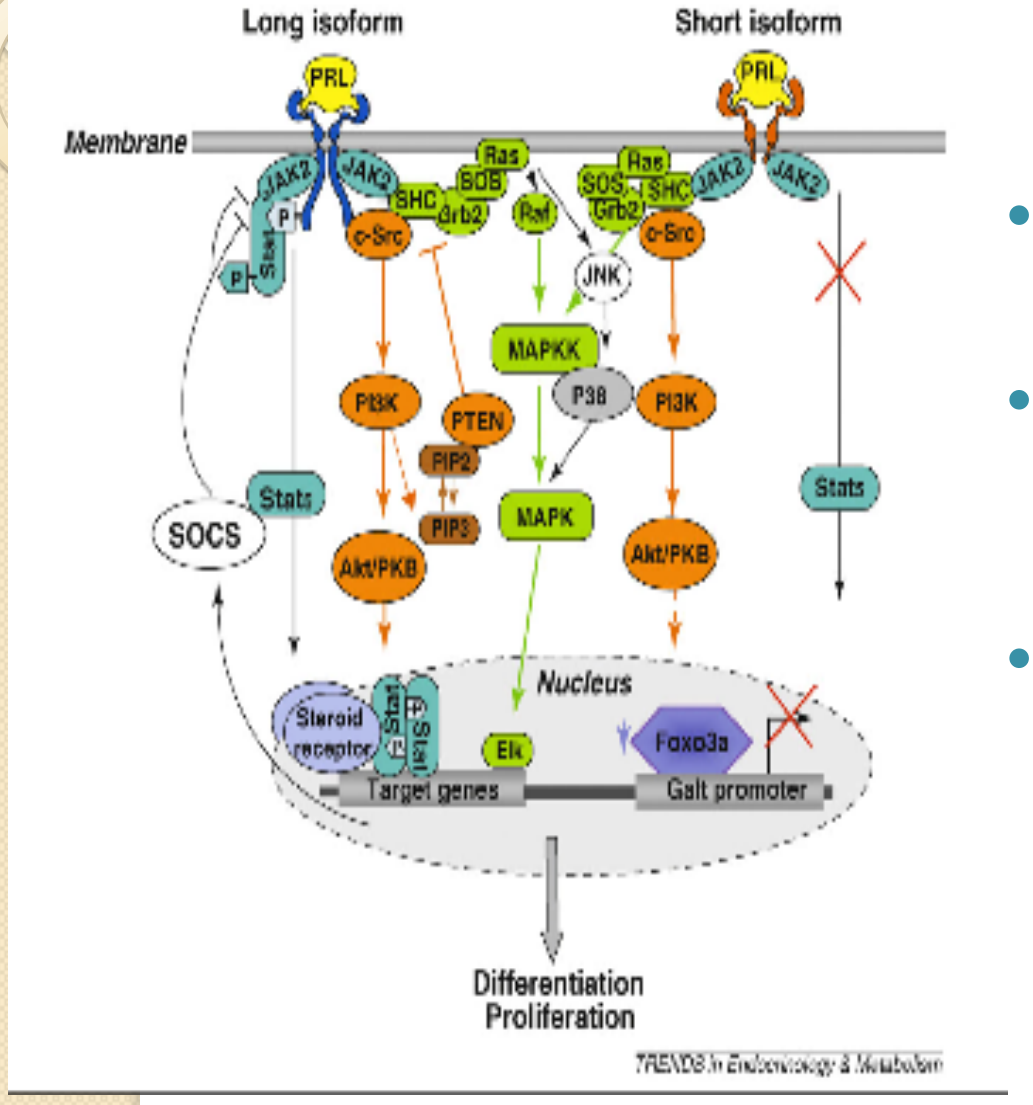
The study thus far...

- Pregnancy stimulates neurogenesis at GD7 and PD7
- Neurogenesis is NOT caused by implantation, estrogen or progesterone
- Effects induced by prolactin mimic those induced by pregnancy
- These effects are specific and selective

Study 5: What is the functional link between neurogenesis and prolactin?

- Prolactin acts through its receptors (Prlr)
  - Co-localization of PRL receptors and sites of neurogenesis would support direct action of prolactin in regulation of neurogenesis
- examined precursor cells *in vitro* for PRL receptors
  - positive results would support cellular rather than environmental effect for PRL

PRL Receptors



Binart *et al.* (2010)

- Structurally similar to growth hormone receptors and EFG Receptor (FGF2)
- Homodimer signaling
  - JAK/STAT pathway
- Multiple PRL receptor isomers
  - alternative splicing
- Short isomer (MAPK only)
  - limited signaling capacity
  - can inhibit long form signaling
  - by formation of heterodimers

Confirming previous studies leads to new findings

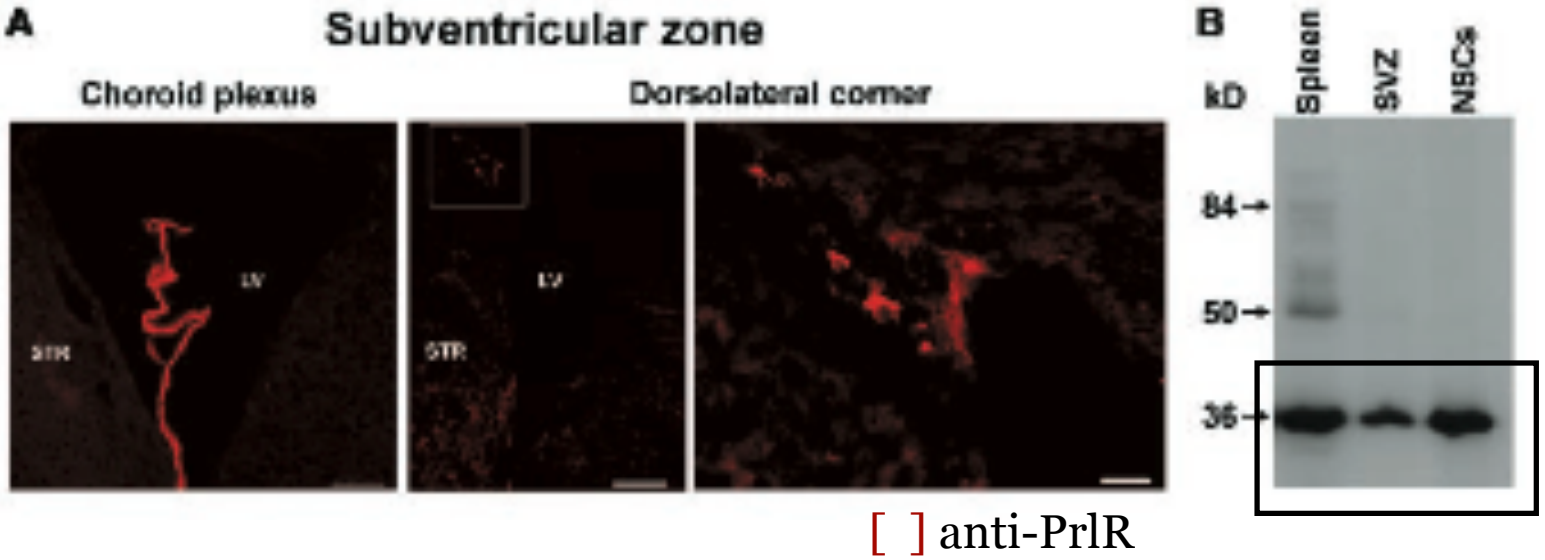
Expected Results:

- PRL Receptor found in choroid plexus
  - Choroid Plexus: cerebrospinal fluid production
- No receptor in Olfactory Bulb

Unexpected Results:

- Prlr also discovered on dorsolateral corner of the SVZ
  - This the departure site for neuronal precursor cells into rostral migratory stream

Localization of PRL Receptor suggests direct role in Cell Proliferation and Migration



[ ] anti-PrlR

- Only the short form (36kD) of PRL Receptor in SVZ
- This Prlr isoform was also found on NSCs in vitro

Conclusion: PRL acts through its receptor directly at the site of neurogenesis and on NSC

Study 6: Effect of PRL on NSC proliferation and differentiation *in vitro*

a) Proliferation
b) Differentiation

Epidermal Growth Factor (EGF)

- 53 amino acid mitogen for adult NSCs
- EGF promotes:
  - SVZ neurogenesis and neuroblast migration into OB (Crag *et al.* 1996)
- NSCs self-renewal and long-term survival in culture requires both:
  1. EGF
  2. Fibroblast Growth Factor (FGF2)

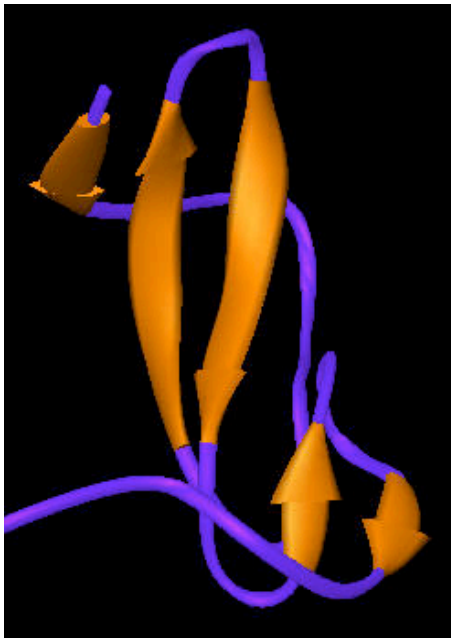
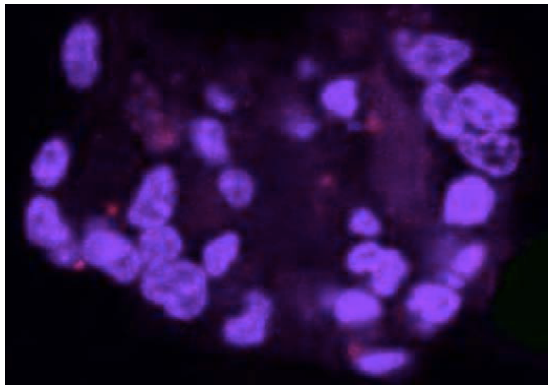


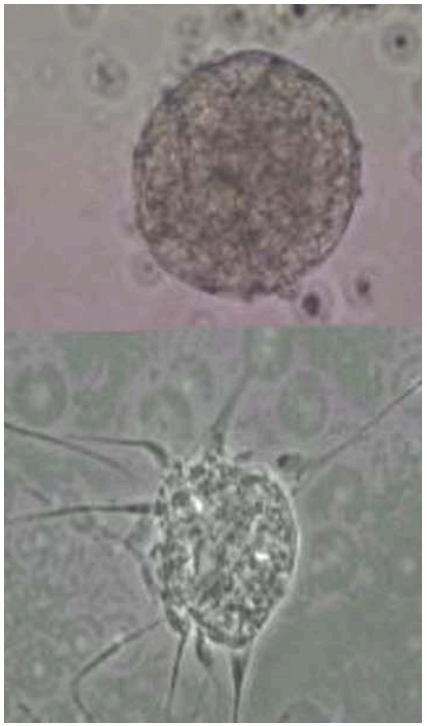
Image: http://www-nmr.cabm.rutgers.edu/photogallery/proteins/gif/3EGF-v3.jpg

Neurospheres

- Free-floating structures generated by NSC cells *in vitro*



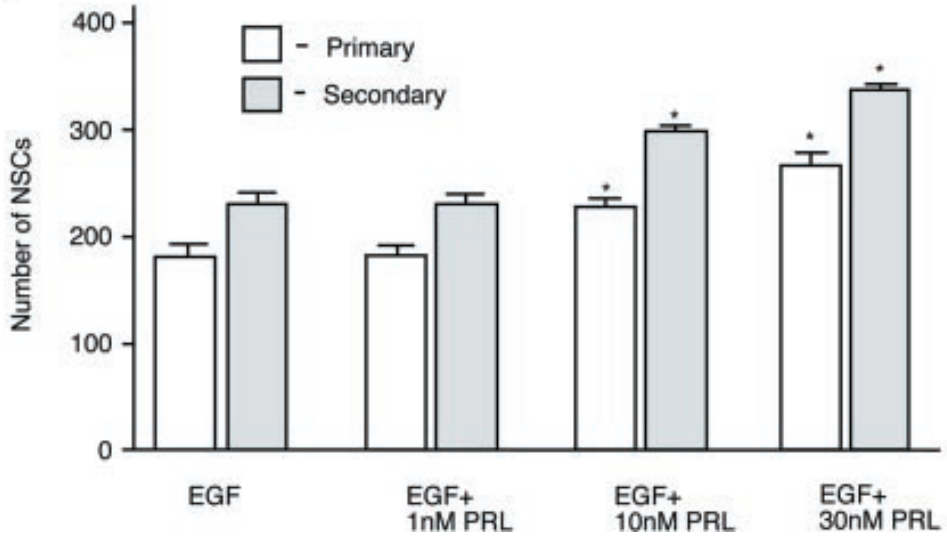
- Morphological and functional heterogeneous
  - Spherical and irregular cell clusters



Study 6a: PRL on NSC proliferation *in vitro*

Results:

- PRL alone added to isolated NSCs did not induce proliferation
- in presence EGF:
  - PRL increased clonally derived neurospheres
  - proliferation and self-renewal of NSCs increased (EGF+PRL)

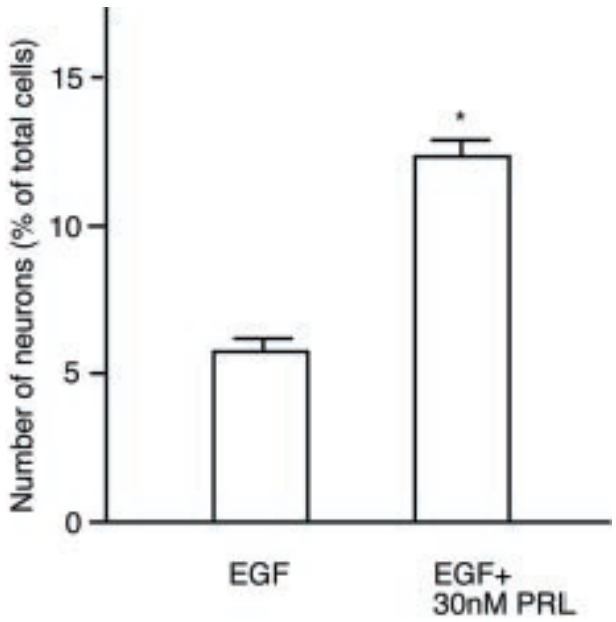


Conclusion: PRL cooperates with EGF to increase proliferation and self-renewal of NSCs

Study 6b: PRL on NSC differentiation

Method: cultured neurospheres in presence of EGF alone or EGF + PRL and counted neurons generated

Results: Neurospheres generated in presence of EGF+PRL produced twice more neurons than in EGF alone



Conclusive findings: PRL can activate NSCs directly to increase their proliferation and differentiation into neurons

Study 7: Is PRL signaling required to mediate neurogenesis?

- Examined mice with a targeted disruption in the gene encoding PRLR
  - Cant use null mutants (Prlr $^{-/-}$)
    - Cannot become pregnant or pseudopregnant
  - Based on previous findings, heterozygous prlr-mutant mice
    - Shows a defect in olfactory-dependent foster pup-induced maternal behaviour
    - Does a 50% of reduction in PRLR affect neurogenesis rates?

Study 7: Continued

- Results:
  - Number of BrdU-labeled cells before mating in both Prlr $^{+/+}$ and Prlr $^{+/-}$ were similar
  - Numbers doubled in mated Prlr $^{+/+}$ females when compared with mated Prlr $^{+/-}$ females

- Concludes:
  - 50% of reduction in BrdU-labeled cells in heterozygous prlr-mutant mice in forebrain SVZ corresponds to the 50% reduction in Prlr gene dose
    - PRL is the mediator of pregnancy-stimulated neurogenesis

Conclusions of Shingo *et al.* (2003)

1. Pregnancy stimulates neurogenesis in SVZ
   - Proliferation, Migration to OB and Differentiation into Neurons
2. Prolactin is required to mediates this neurogenesis
   - other pregnancy-mediated changes ruled out and also increases neurogenesis in males
   - prolactin signals through short form receptors in the SVZ (on NSCs in vitro too!)
   - PrIr -/+ mutants: evidence for dose dependence on PrIr for neurogenesis during pregnancy

What is the significance of Shingo *et al*'s paper?

- This was the first study to show that rates of neurogenesis can be altered by changes in the endocrine state of an animal
- Evidence that PRL plays additional roles in the CNS to facilitate adaptive maternal behaviours than initially supposed
  - short form of the PRL Receptor may have a distinctive signaling pathway due to its localization
  - PRL may be functionally distinct from PL in the adult brain
- Intriguing results: PRL increases neurogenesis in both males and females
  - stimulate interest to examine other states where [PRL] increases: mating season, pathologies (schizophrenia)

Strengths

I. Methodical structure
II. Thorough: Used double methods to ensure accuracy of results, and confirmed results of previous studies
III. Concise and condensed

Weakness and Limitation

- Methods not extensively described
- Technological restrictions-did not test the functionality of neurons
- Discussion not explained in depth in some experiments
- Did not make explicit connection to human neurogenesis

Much has changed since 2003...

- There have been significant strides in model systems, genetic manipulation and computing technology:
  - Production of inducible knockouts and chimeras to upregulate or downregulate neurogenesis
  - Computer modelling to determine functions of new neurons
- New methodologies, as well as time, have led to significant breakthroughs

Discussion of newer research

1. Roles of prolactin in the CNS
2. Induction of neurogenesis

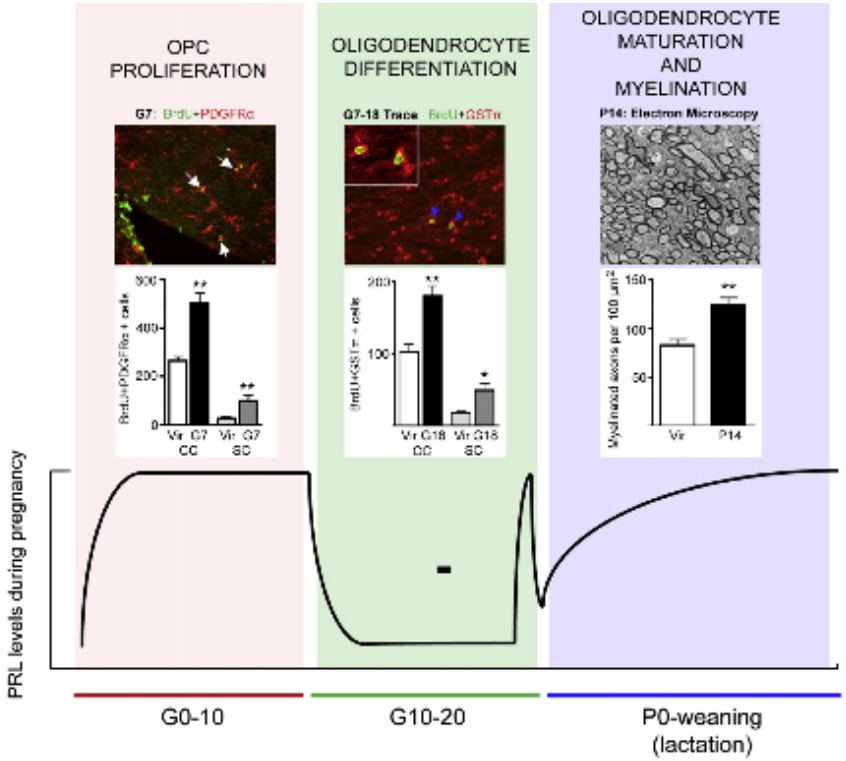
In the last 7 years: Prolactin and Neurogenesis

- Prolactin: a pleiotropic hormone
- Roles in neurogenesis and induction of maternal behaviour, but also involved in
  - modulating stress-response
    - (Torner et al, 2009)
  - increasing remyelination of dendrites
    - (Gregg et al, 2007)

Re-myelination and PRL

- Recall: SVZ NSCs → Oligodendrocytes (OPC)
  - Immature oligodendrocytes (re)myelinate neurons
- Demyelination: pathologies like Multiple Sclerosis
  - Correlation: MS remission during preganacy

- SVZ neurogenesis ***<u>can</u>*** enhance remyelination
  - Nait-Oumesmar *et al.* (2007)
  - Gregg et al. (2007)



Recent studies on the induction of neurogenesis

- Mak *et al.* (2007) showed that pheromones increase SVZ and dentate gyrus cell proliferation in mice
- Furuta and Bridges (2009) study showed that maternal behavior itself, independent of pregnancy and lactation, stimulated neurogenesis in the SVZ

Future Directions

I. Great interest in neurogenesis:
   - replace neurons lost to injury
   - neurodegenerative disease

II. Stroke Recovery and Therapy
   - neurogenesis in stroke recovery (Chopp *et al.* 2007)
   - progenitors from SVZ migrate to injury site and differentiate into neurons (Yamashita *et al.* 2006)
   - PRL as a potential therapeutic agent-redirected SVZ neurogenesis (Arvidsson *et al.* 2002)

III. Role in Aging
   - Age-related declines in both olfactory ability and hippocampal-mediated behavours not fully examined

Future Research

Upon recognition of ongoing adult neurogenesis, many important questions still remain:

1. Functional significance and evolutionary advantages of adult neurogenesis in OB or hippocampus
2. Why progenitors in SVZ and SGZ continue to proliferate and produce new neurons and not anywhere else?
3. Signaling mechanism of ongoing OB neurogenesis very complex and are only partially understood

Conclusion

- Neurogenesis occurs throughout adulthood
- The complexity of its regulation is only beginning to be comprehended
- Neurogenesis in pregnancy is adaptive: it improves olfactory discrimination necessary for maternal behaviours
- Prolactin is a pleiotrophic hormone that through its receptor can modulate cell proliferation in the adult mammalian brain
- There are still so many fundamental questions to answer before harnessing adult neurogenesis to combat disease will be possible