Brain science and Brain Disease
Class #2
Outline for Today

• An introduction to brain cells (neurons) and connections (synapses) and chemical neurotransmitters
• What is depression?
• The connection between depression and serotonin
• New ideas about the biological basis of depression
A brain without stain
Nissl stain labels neuronal cell bodies

Figure 13. Nissl stain of the visual cortex reveals the different layers I through VI quite clearly.
Nissl Stain defines Brodman’s areas
Brodmann's areas
Cerebral Cortex Is Organized in Layers

- Unstained brain has very low contrast, making structure hard to see.
- Nissl staining reveals cell bodies
- Golgi staining labels entire cells
- The distinctive laminar structure of cell packing in the cortex can be seen using these staining techniques.
Synapses

- The connections between neurons are called synapses
- Each neuron may make ~ 10,000 synapses with other neurons
• http://vimeo.com/38852446
Golgi Stain shows cell bodies and processes

Golgi stain is a silver-based stain that is used to label a (small) random subset of neurons.

Spines – found on dendrites
Golgi stained mouse brain
Golgi stain
Cajal and Golgi

**Camillo Golgi**

Developed staining technique that allowed complete structure of neurons to be visualized. Believed that neurons were physically connected, forming a “reticulum”.

**Santiago Ramon y Cajal**

Believed that neurons were individual cells connected to each other by chemical transmission. The term “synapse” was later coined by Sherrington.

Cajal and Golgi shared the Nobel Prize in 1906 – “in recognition of their work on the structure of the nervous system".

Debate was not completely resolved until 1950s with advent of electron microscopy.
Depression
DSM IV
Major Depressive Episode

• A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.

• Note: Do note include symptoms that are clearly due to a general medical condition, or mood-incongruent delusions or hallucinations.

• (1) depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful). Note: In children and adolescents, can be irritable mood.

• (2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others).

• (3) significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. Note: In children, consider failure to make expected weight gains.

• (4) insomnia or hypersomnia nearly every day

• (5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)

• a general medical condition.
(6) fatigue or loss of energy nearly every day

(7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)

(8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)

(9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

B. The symptoms do not meet criteria for a Mixed Episode.

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

D. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).

E. The symptoms are not better accounted for by Bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.
- Major Depressive Disorder
- Single Episode
  - A. Presence of a single Major Depressive Episode
  
  - B. The Major Depressive Episode is not better accounted for by Schizoaffective Disorder and is not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Psychotic Disorder Not Otherwise Specified.
  
  - C. There has never been a Manic Episode, a Mixed Episode, or a Hypomanic Episode. Note: This exclusion does not apply if all the manic-like, mixed-like, or hypomanic-like episodes are substance or treatment induced or are due to the direct physiological effects of a general medical condition.

- Recurrent
  
  - A. Presence of two or more Major Depressive Episodes.
  
  - B. The Major Depressive Episodes are not better accounted for by Schizoaffective Disorder and are not superimposed on Schizophrenia, Schizophreniform Disorder, Delusional Disorder, or Psychotic Disorder Not Otherwise Specified.
  
  - C. There has never been a Manic Episode, a Mixed Episode, or a Hypomanic Episode. Note: This exclusion does not apply if all the manic-like, mixed-like, or hypomanic-like episodes are substance or treatment induced or are due to the direct physiological effects of a general medical condition.
Depression videos
Depressing statistics

- Anxiety and depression affect over 20% of the population at some point in their lifetime.
- The treatment and direct consequences of anxiety disorders costs $44 billion per year in US
- The treatment and direct consequences of depression costs $80 billion per year in US

Total NIH Budget = $35B/year
NIH Budget for neuroscience = $5.5B/year
NIMH budget = ~$2.2B/year
NIH budget for depression = $419M/year
Cost of bringing a new drug to market = $1.8B
History of treatments for depression

- Major Depressive Disorder identified in 1970s

- Iproniazid (antituberculosis drug, actually MAOi inhibitor) first identified a improving mood – use discontinued due to side effects.
- Raudixin (anti-hypertensive) decreased serotonin levels and caused depression
- Tricyclics (imipramine)
- 1980s SSRIs (prozac, aka fluoxitine) introduced

- Electroconvulsive therapy (ECT) is still the best treatment for many patients
Serotonin
Major classes of antidepressants

**Mono Amine Oxidase Inhibitors (MAOIs):**
- Phenylzine
- Isocarboxazid

**Tricyclics:**
- Imipramine (Antideprin, Tofranil)
- Amitriptyline (Elavil)

**Selective Serotonin Reuptake Inhibitors**
- Fluoxetine (Prozac)
Genetics of depression

- Twin studies show that 30-40% of variance in occurrence of depression/anxiety can be attributed to genetic variation.

- Adoption Studies:
  - Suicide rates of biological parents of depressed, adopted children are 6X higher than suicide rates of non-depressed adopted children

- Potential Genes:
  - Promoter for 5HT Transporter
  - Chromosome 18 (18q22-23)
  - Chromosome 17 (17q11.1-q12), a serotonin transporter
  - Mutations in MAO (degrades serotonin)
Steps in serotonin transmission

- Synthesis
- Packaging
- Release
- Postsynaptic action
- Re-uptake
- Degradation
- Recycling

*Fig. 1 Schematic illustration of 5-HTP conversion to Serotonin and mechanism of SSRIs. 1: Tryptophan enters system. 2: Converts to 5-HTP. 3: 5-HTP converts to 5-HT (serotonin). 4: 5-HT passes through membrane into synaptic cleft. 5: 5HT stimulates its receptor. 6: 5-HT joins with reuptake transporter to cycle through again. 7: Reuptake inhibitors (such as Prozac™) increase 5-HT level in synaptic cleft by inhibiting the reuptake of 5-HT by the presynaptic neuron. SSRIs=Selective Serotonin Reuptake Inhibitors.*
The Serotonin Neuron

serotonin

serotonin receptor

The Serotonin Neuron

Interlude: the serotonergic system

A

serotonin

B

C

Fig. C from Hariri and Holmes (2006)
How effective are antidepressants?

- Results showed that at the end of the 8-week trial, approximately twice as many patients treated with Effexor XR experienced full remission, based on the HAM-D score, compared with those treated with Prozac or placebo (37% vs 22% and 18%, respectively, $P < .05$). In addition, when compared with patients treated with Prozac or placebo, more patients treated with Effexor XR demonstrated symptomatic improvements, based on the total MADRS score (58% vs 51% and 39%, respectively) and the CGI improvement score (71% vs 62% and 52%, respectively).

- *Journal of Affective Disorders* 2000
Serotonin and Depression: A Disconnect between the Advertisements and the Scientific Literature

Jeffrey R. Lacasse, Jonathan Leo

<table>
<thead>
<tr>
<th>Quotation</th>
<th>Source</th>
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<tbody>
<tr>
<td>“Although it is often stated with great confidence that depressed people have a serotonin or norepinephrine deficiency, the evidence actually contradicts these claims” [50].</td>
<td>Professor Emeritus of Neuroscience Elliot Valenstein, in <em>Blaming the Brain</em> (1998), which reviews the evidence for the serotonin hypothesis.</td>
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<td>“Given the ubiquity of a neurotransmitter such as serotonin and the multiplicity of its functions, it is almost as meaningless to implicate it in depression as it is to implicate blood” [11].</td>
<td><em>Science writer John Horgan</em>, in his critical examination of modern neuroscience, <em>The Undiscovered Mind</em> (1999).</td>
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<td>“A serotonin deficiency for depression has not been found” [51].</td>
<td>Psychiatrist Joseph Glenmullen, clinical instructor of psychiatry at Harvard Medical School, in <em>Prozac Backlash</em> (2000).</td>
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<td>“So far, there is no clear and convincing evidence that monoamine deficiency accounts for depression; that is, there is no “real” monoamine deficit” [44].</td>
<td>Psychiatrist Stephen M. Stahl, in a textbook used to teach medical students about psychiatric medications, <em>Essential Psychopharmacology</em> (2000).</td>
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<td>“Some have argued that depression may be due to a deficiency of NE [norepinephrine] or 5-HT [serotonin] because the enhancement of noradrenergic or serotoninergic neurotransmission improves the symptoms of depression. However, this is akin to saying that because a rash on one’s arm improves with the use of a steroid cream, the rash must be due to a steroid deficiency” [52].</td>
<td>Psychiatrists Pedro Delgado and Francisco Moreno, in “Role of Norepinephrine in Depression,” published in the <em>Journal of Clinical Psychiatry</em> in 2000.</td>
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<td>“…I wrote that Prozac was no more, and perhaps less, effective in treating major depression than prior medications…. I argued that the theories of brain functioning that led to the development of Prozac must be wrong or incomplete” [53].</td>
<td>Brown University psychiatrist Peter Kramer, author of <em>Listening to Prozac</em>, which is often credited with popularizing SSRI s, in a clarifying letter to the <em>New York Times</em> in 2002.</td>
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<td>“I spent the first several years of my career doing full-time research on brain serotonin metabolism, but I never saw any convincing evidence that any psychiatric disorder, including depression, results from a deficiency of brain serotonin. In fact, we cannot measure brain serotonin levels in living human beings so there is no way to test this theory. Some neuroscientists would question whether the theory is even viable, since the brain does not function in this way, as a hydraulic system” [54].</td>
<td>Stanford psychiatrist David Burns, winner of the A.E. Bennett Award given by the Society for Biological Psychiatry for his research on serotonin metabolism, when asked about the scientific status of the serotonin theory in 2003.</td>
</tr>
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<td>“Indeed, no abnormality of serotonin in depression has ever been demonstrated” [55].</td>
<td>Psychiatrist David Healy, former secretary of the British Association for Psychopharmacology and historian of the SSRIs, in <em>Let Them Eat Prozac</em> (2004).</td>
</tr>
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<td>“We have hunted for big simple neurochemical explanations for psychiatric disorders and have not found them” [56].</td>
<td>Psychiatrist Kenneth Kendler the coeditor-in-chief of <em>Psychological Medicine</em>, in a 2005 review article.</td>
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<tr>
<td>Medication</td>
<td>Selected Content from Consumer Advertisement</td>
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<td>Citalopram</td>
<td>“Celexa helps to restore the brain’s chemical balance by increasing the supply of a chemical messenger in the brain called serotonin. Although the brain chemistry of depression is not fully understood, there does exist a growing body of evidence to support the view that people with depression have an imbalance of the brain’s neurotransmitters” [57].</td>
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<td>Escitalopram</td>
<td>“LEXAPRO appears to work by increasing the available supply of serotonin. Here’s how: The naturally occurring chemical serotonin is sent from one nerve cell to the next. The nerve cell picks up the serotonin and sends some of it back to the first nerve cell, similar to a conversation between two people. In people with depression and anxiety, there is an imbalance of serotonin—too much serotonin is reabsorbed by the first nerve cell, so the next cell does not have enough, as in a conversation, one person might do all the talking and the other person does not get to comment, leading to a communication imbalance. LEXAPRO blocks the serotonin from going back into the first nerve cell. This increases the amount of serotonin available for the next nerve cell, like a conversation moderator. The blocking action helps balance the supply of serotonin, and communication returns to normal. In this way, LEXAPRO improves symptoms of depression” [58].</td>
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<td>Fluoxetine</td>
<td>“When you’re clinically depressed, one thing that can happen is the level of serotonin (a chemical in your body) may drop. So you may have trouble sleeping. Feel unusually sad or irritable. Find it hard to concentrate. Lose your appetite. Lack energy. Or have trouble feeling pleasure…to help bring serotonin levels closer to normal, the medicine doctors now prescribe most often is Prozac®” [59].</td>
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<td>Paroxetine</td>
<td>“Chronic anxiety can be overwhelming. But it can also be overcome…Paxil, the most prescribed medication of its kind for generalized anxiety, works to correct the chemical imbalance believed to cause the disorder” [60].</td>
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<td>Sertraline</td>
<td>“While the cause is unknown, depression may be related to an imbalance of natural chemicals between nerve cells in the brain. Prescription Zoloft works to correct this imbalance. You just shouldn’t have to feel this way anymore” [5].</td>
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</tbody>
</table>
• Little or no correlation between measured serotonin levels and mood
• Increasing serotonin levels does not improve mood
• Decreasing serotonin levels does not yield depression in normal individuals
  – Though it may make depression worse in some depressed patients
• Many anti-depressants have non-specific effects
• Some anti-depressants are unlikely to influence serotonin at all
A puzzle:

Antidepressants typically take several weeks to reach full efficacy. Presumably, the specific targets of SSRIs are affected much sooner than this. Why is there such a long lag time for antidepressants to work?

Hints:

What kinds of recovery processes in the brain might occur on the scale of weeks?

What are some of the gross changes in brain structure observed in depressive individuals?
Animal models for depression

• Learned helplessness
• BDNF knockout
• Amphetamine withdrawl
Adult brains (humans and other animals) are capable of producing new neurons. This phenomenon, known as neurogenesis, occurs in two brain regions: the subventricular zone (SVZ), and the subgranular zone (SGZ).

New neurons generated in the subgranular zone supply new neurons to the hippocampus.

Could the reduction of brain volume and brain deterioration in depression be the result of a decrease in neurogenesis?
Is my mouse depressed?

- Novelty suppressed feeding
- Tail suspension test
Evidence that antidepressants work by increasing neurogenesis

Is neurogenesis necessary for antidepressants to work, or does it just accompany recovery?

History of adult neurogenesis

- Cajal claimed that neurons did not divide except in neonatal animals
- In the 1960s tritiated thymidine was used to label new neurons (thymidine was incorporated into DNA of dividing cells) – Joseph Altman
- Dismissed as being an artifact glia-genesis rather than neurogenesis - Pasko Rakic
- BrdU (Bromo deoxy-uridine) nucleotide analog against which antibodies can be raised
- This allowed two color fluorescent immunolabeling to determine whether new cells were also stained for markers of neurons
- These techniques have identified two areas of constitutive adult neurogenesis
Where are new neurons made?
Sources of new neurons

• Periventricular astrocytes (B cells) are thought to be the neural stem cells.
• These divide to generate transit amplifying cells (C cells)
• Which then generate neuroblasts (A cells) which migrate up the RMS to the OB
  – But some cells may be still dividing or at least multipotent during this journey.
<table>
<thead>
<tr>
<th>Principle</th>
<th>Based on incorporation of nucleotide analogs</th>
<th>Based on genetic marking by retroviruses</th>
<th>Based on expression of specific markers</th>
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<td>Imaging</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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**Birth dating**
- Good
- Good
- Poor

**Tracing**
- Permanent
- Permanent
- Transient / Permanent

**Cell Population**
- Whole population
- Limited cells
- Whole population

**Visualization**
- Fixation / Processing
- Direct
- Processing / Direct

**Morphology**
- Nuclear
- Whole cell
- Cellular / Whole cell

**Concerns**
- DNA repair
- Invasive
- Specificity

Ming, G and Song, H. 2005
A novel method for analysis of adult neurogenesis

Au and Fishell et al 2006
Olfactory bulb

Ming, G and Song, H. 2005
Annual Rev. Neurosci. 28: 223–50
## Saving neurons? Activity-dependent effects on Neurogenesis

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Effect on OB NG</th>
<th>Effect on DG NG</th>
<th>Stage affected</th>
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<tbody>
<tr>
<td>Odor deprivation</td>
<td>-30 (GCs)% [8,30,32]</td>
<td>0%</td>
<td>Survival and prolif.</td>
</tr>
<tr>
<td>Odor enrichment</td>
<td>+20 (GCs)% [33,34]</td>
<td>0% [33]</td>
<td>Survival</td>
</tr>
<tr>
<td>Odor discrim. training</td>
<td>+70% (GCs)</td>
<td>0%</td>
<td>Survival</td>
</tr>
<tr>
<td>Wheel Running</td>
<td>0%</td>
<td>+120% [35]</td>
<td>Proliferation and survival</td>
</tr>
<tr>
<td>Enriched environment</td>
<td>0%</td>
<td>+80 [11,36]</td>
<td>Survival</td>
</tr>
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Hippocampus
Make a new neuron type
Allman’s von Economo neuron

Fig. 1. Photomicrographs of soma and proximal dendrites of (a) a pyramidal and (b) the VENs stained with the Golgi method. Photomicrographs are montages taken of several planes and/or fields of view. Scale bar applies to both images.
http://www.allmanlab.caltech.edu/PDFs/WatsonGolgi2006.pdf
Grow a brain
Grow a Brain
Brain size scales with body size
Add brain areas