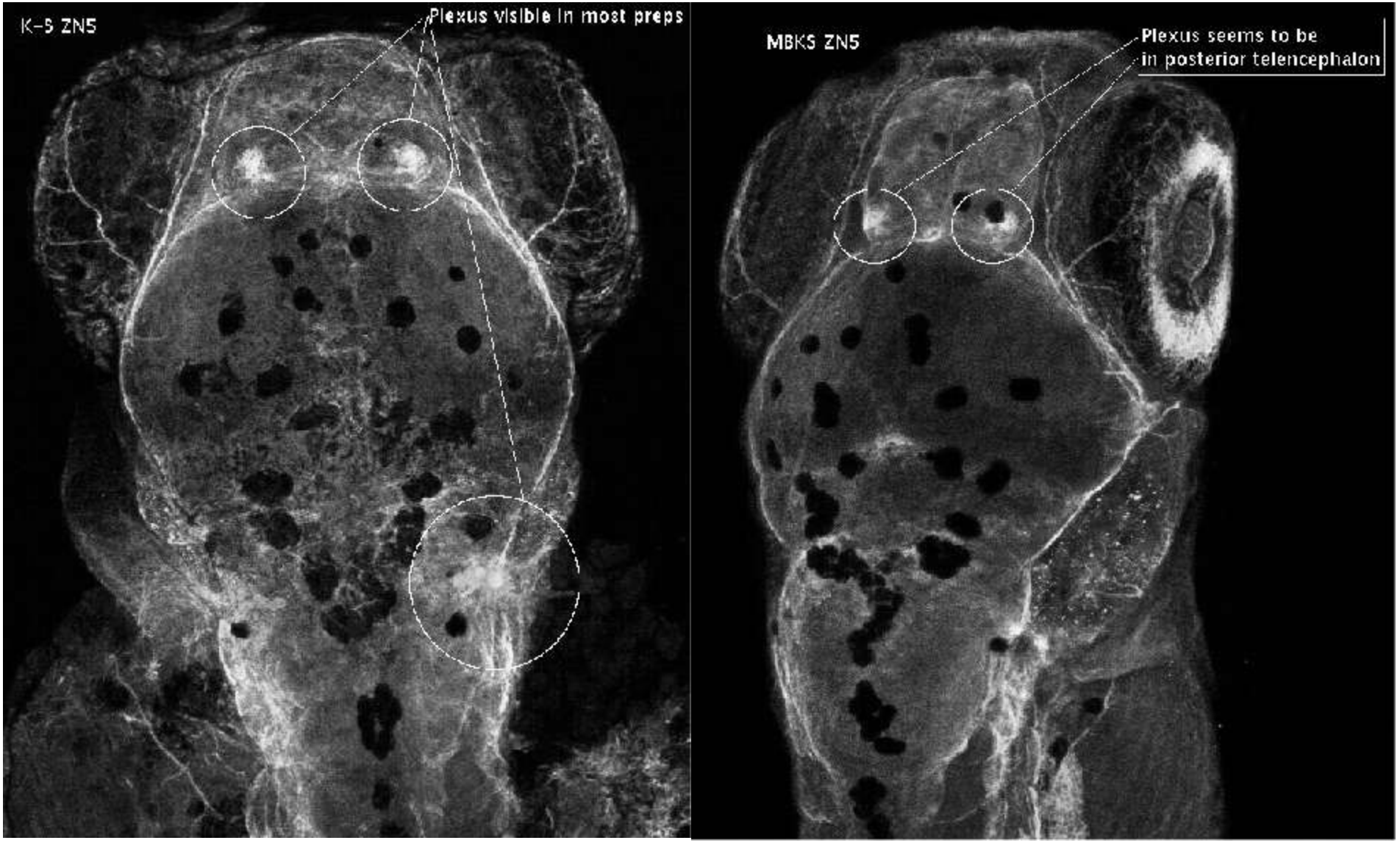
COVER A late-stage *Xenopus tropicalis* (western clawed frog) tadpole, about 5 centimeters in length, with emerging hindlimbs. Through the skin of the head, the optic, trigeminal, and olfactory nerves, as well as the paired thymus and large blood vessels, are visible. A Report on page 633 describes the genome of this organism, which is an important model for vertebrate development. Photo: Siwei Zhang, Jingjing Li, Enrique Amaya


K-5 ZN5
Plexus visible in most preps
MBK5 ZN5
Plexus seems to be
in posterior telencephalon

Biol 580, Cellular Neuroscience, Spring 2010, Exam 4. The exam will be held at the assigned final exam time, Monday May 10th from 10:20-12:10, in the regular classroom.

General instructions: This is an oral exam. Prepare a ~10 minute presentation using hand drawn visual aids only that addresses one of the questions below.

Learning objective: To apply your knowledge about cellular neuroscience to a novel, hypothetical research challenge or topic.

Evaluation: Presentations will be scored on a scale of 1-15 by the faculty members present. Evaluation will be based on 1. clarity / comprehensibility of ideas, 2. scientific sophistication, 3. adherence to specific guidelines.

General requirements for answers:

An explicit connection to a topic we covered in class

Citation of 1-2 cellular neuroscience related research articles

Drawing should at minimum illustrate an anatomical feature and/or physiological process/es within neurons, and/or a mathematical model of a neuronal process.

Specific questions- select ONE to answer:

1. What is the 'dark matter' of cellular neuroscience, ie, what do you think is the most important unresolved questions about how neurons process information, and why? B. How would you start to address that question?

2. Imagine a race of super-intelligent humanoid space aliens visited earth and offered themselves as subjects for invasive, acute experimentation. What would you want to know about the functioning of the putative neurons in their putative nervous systems, and why? B. What cellular neuroscience research methods would this require?

3. We paid glial cells relatively little attention in this course. Describe one way in which glial cells influence information processing in neurons. Make the case that glial cells deserve to receive more attention in a cellular neuroscience course.

4. Select a cellular neuroscience phenomenon. A. Explain the phenomenon, and B. explain something about it at each of the following levels of analysis: behavioral, systems, circuit, cell biological, genetic, computational

■ HYPOTHESIS

Cognitive Role of Neurogenesis in Depression and Antidepressant Treatment

TARIQUE D. PERERA, SUNGSHIC PARK, and YELENA NEMIROVSKAYA
Department of Psychiatry, College of Physicians and Surgeons, Columbia University Medical Center, and the New York State Psychiatric Institute, New York, New York

The discovery of newborn neurons in the adult brain has generated enormous interest over the past decade. Although this process is well documented in the hippocampus and olfactory bulb, the possibility of neuron formation in other brain regions is under vigorous debate. Neurogenesis within the adult hippocampus is suppressed by factors that predispose to major depression and stimulated by antidepressant interventions. This pattern has generated the hypothesis that impaired neurogenesis is pathoetiological in depression and stimulation of newborn neurons essential for effective antidepressant action. This review critically evaluates the evidence in support of and in conflict with this theory. The literature is divided into three areas: neuronal maturation, factors that influence neurogenesis rates, and function of newborn neurons. Unique elements in each of these areas allow for the refinement of the hypothesis. Newborn hippocampal neurons appear to be necessary for detecting subtle environmental changes and coupling emotions to external context. Thus speculatively, stress-induced suppression of neurogenesis would uncouple emotions from external context leading to a negative mood state. Persistence of negative mood beyond the duration of the initial stressor can be defined as major depression. Antidepressant-induced neurogenesis therefore would restore coupling of mood with environment, leading to the resolution of depression. This conceptual framework is provisional and merits evaluation in further experimentation. Critically, manipulation of newborn hippocampal neurons may offer a portal of entry for more effective antidepressant treatment strategies. NEUROSCIENTIST 14(4):326–338, 2008.
DOI: 10.1177/1073858408317242

KEY WORDS *Neurogenesis, Proliferation, Depression, Hippocampus, Antidepressants, Cognition*

Where does adult neurogenesis happen in the human brain?

How is neurogenesis demonstrated?

BrdU pulse→
few weeks for incorporation→
fixation→
colabel with mature-neuron-specific marker (NeuN)→
secondary antibodies→
confocal colocallization

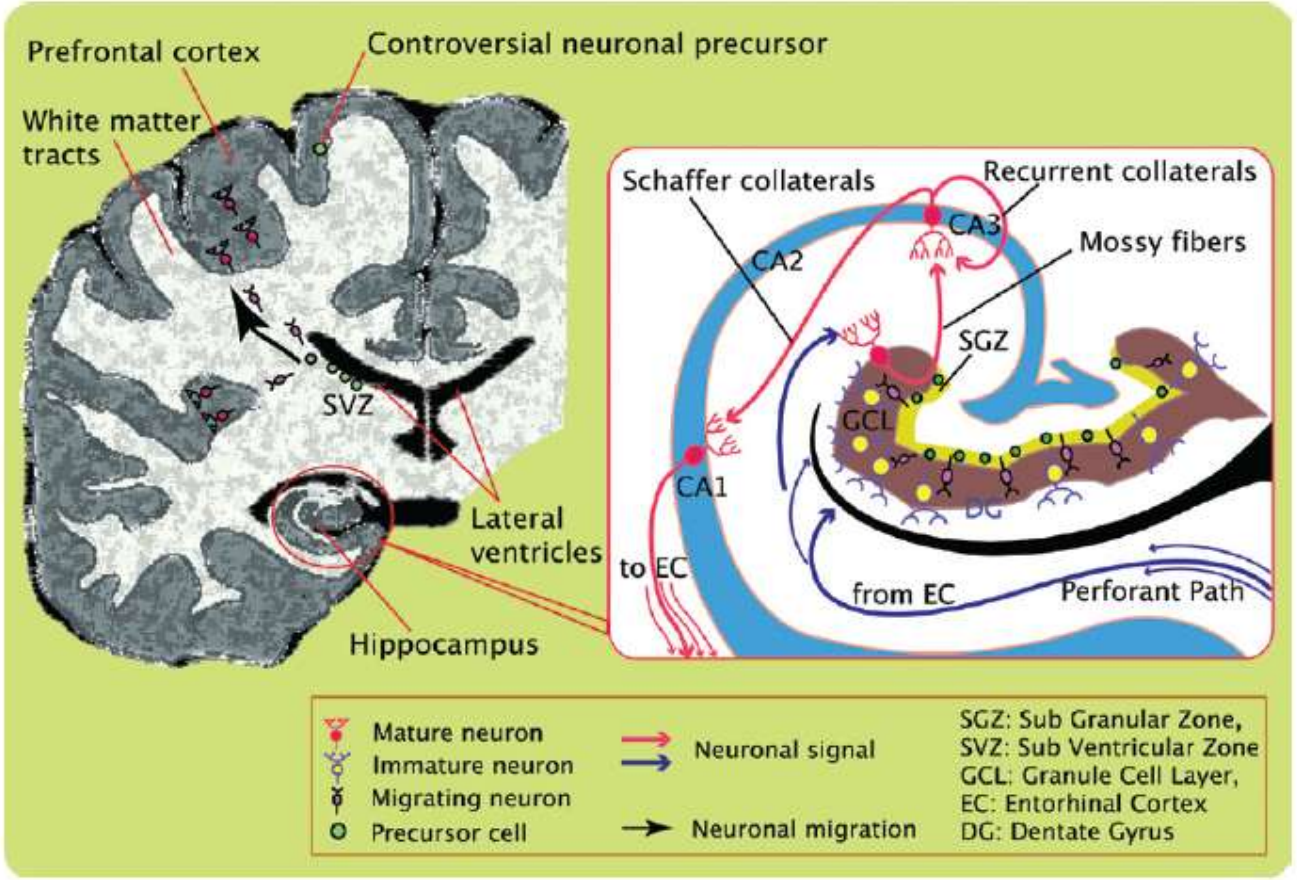
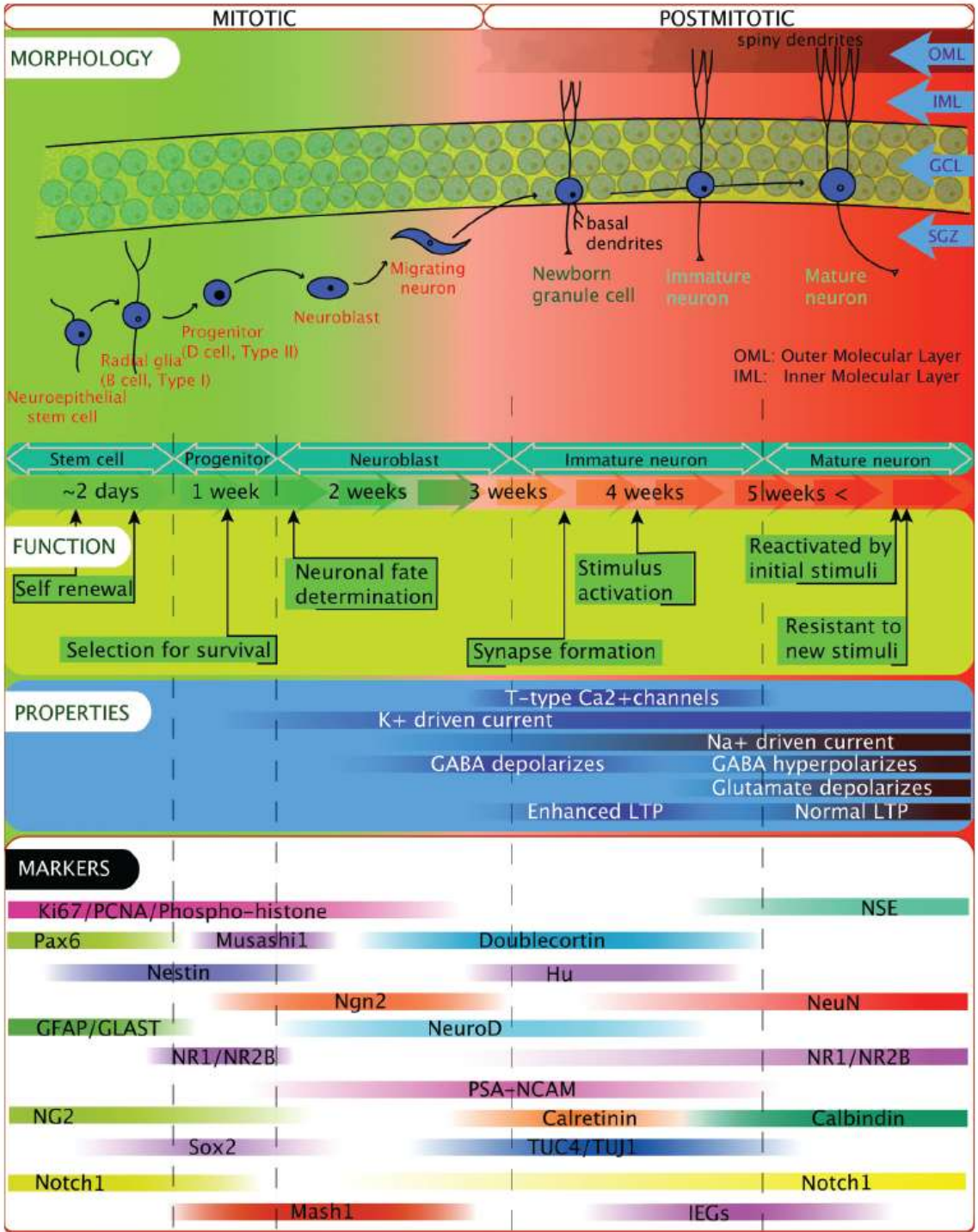


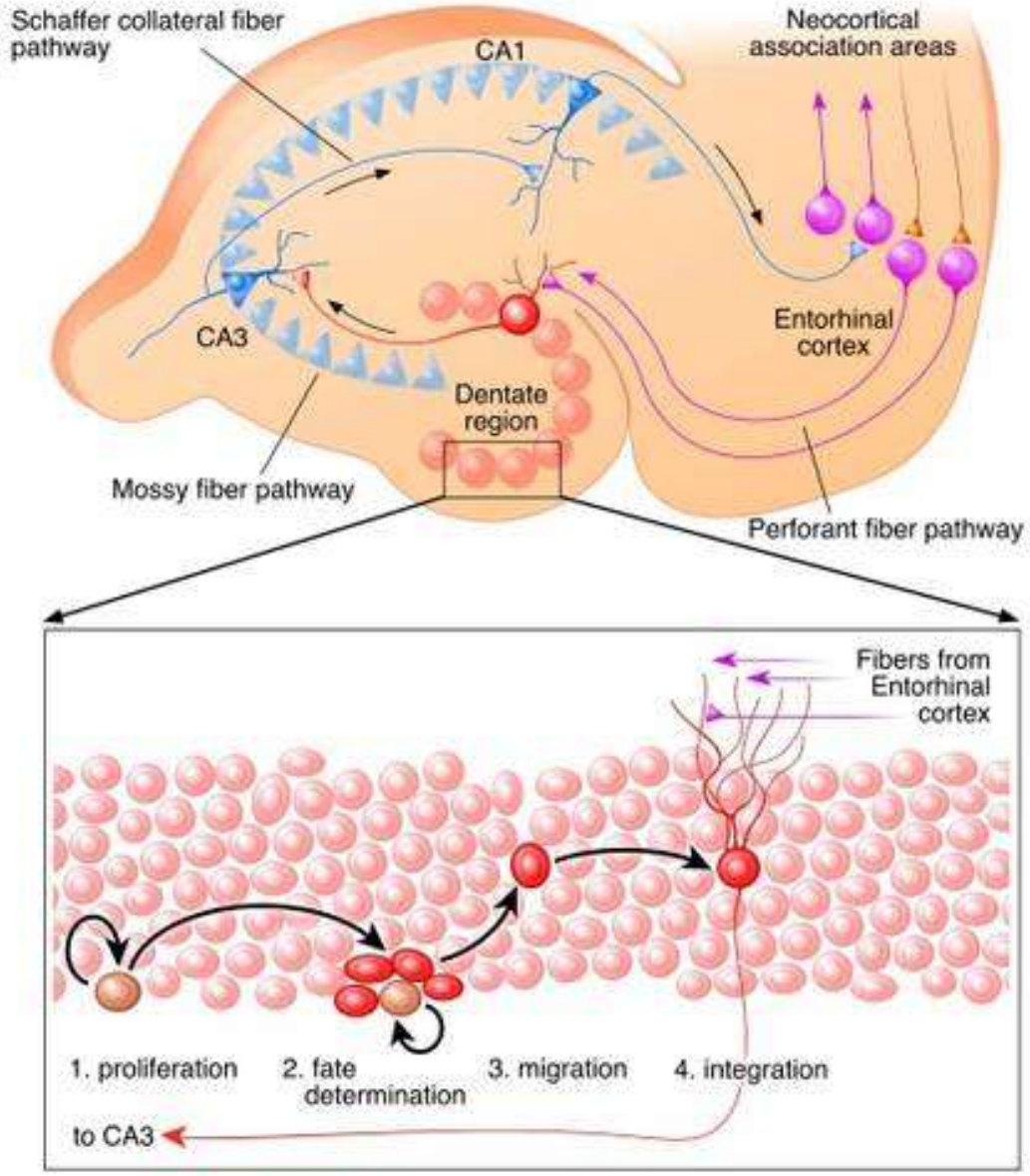
Fig. 1. Neurogenesis in the hippocampus and cortex.

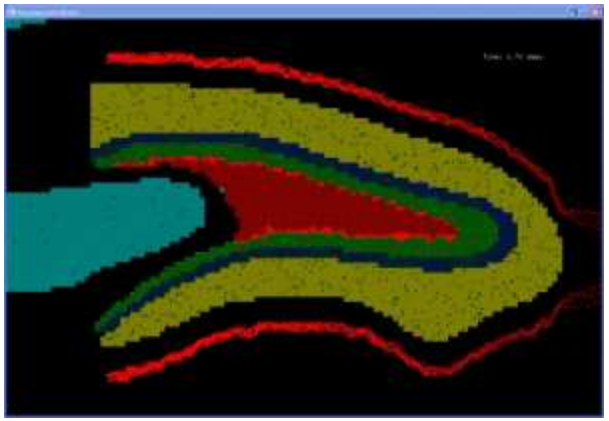


Neuron maturation in dentate gyrus. Stages:

1. Stem cells: multipotent, capable to long term self renewal via non symmetrical division. Called radial glia in adult brain, stain positive for glial markers, GFAP. Can generate all 3 main neural populations: astroglia, oligodendrocytes, neurons. (Tissue specific stem cell were hard for Friday's speaker to explain since micoRNA switches from dedicated to pluripotent)
2. Progenitor: appear 1-2 weeks post mitosis, stop expressing GFAP. Plump morphology. Outside stimuli (exercise, learning, antidepressant drugs) can promote neural over glia fate.
3. Neuroblast: first irreversible turn toward neuron fate. Promoted by BDNF, possibly through activity dependent mechanism. Stress steers progenitors away from turn toward neuroblast. Markers: NeuroD, Neurogenin
4. Migrating neurons: elongated shape, expression of neuron, guidance, adhesion markers. Has short trip from SGZ in hippocampus, but a long trip from SVZ to cortex
5. Newborn granule cells: GABA depolarizes, presence of basal dendrites
6. Immature neurons: loose basal dendrites, extend apical dendrites, begins responding to environmental stimuli, hyperexcitable, better LTP than mature neurons, possibly through increased expression of NRB2 subunit.
7. Mature neurons: GABA becomes hyperpolarizing, less activation by novel environmental stimuli, downregulation of NRB2 NMDA subunit, can be activated by associated stimuli (memory), loss of t-type Ca++ channels, elaboration of dendrites and spines. Time delay for new neurons to become functional (~2-4 weeks) corresponds to the delay of antidepressant efficacy on mood
8. Other fates besides granule cells: glia (astro, mico, oligo), blood vessels. Stress / depression / anxiety promote these fates in exchange for neuronal fate. This is correlated with a decrease in hipp volume in patients and a decrease in spatial memory and other hipp dependent cognition

Additional view of dentate gyrus neurogenesis

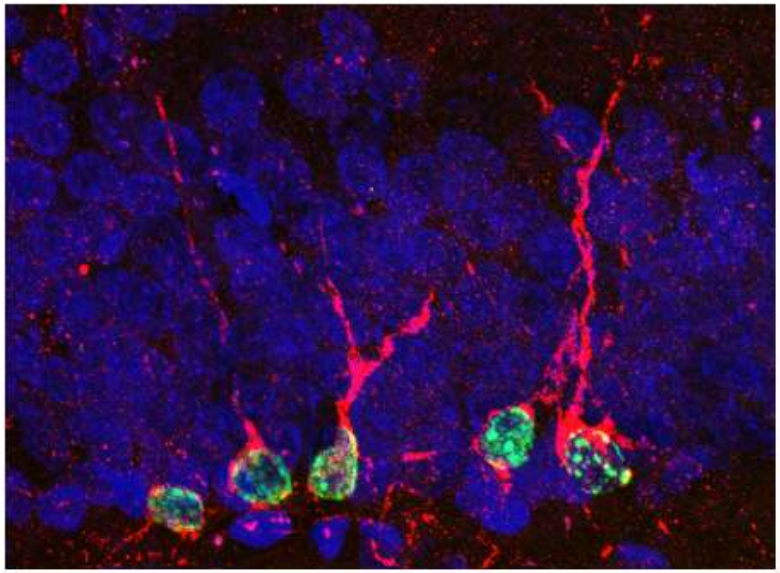




http://www.itee.uq.edu.au/~milford/index.html

About Us
Faculty
Areas of Research Interest
Seminars
Interest Groups
Training Opportunities
Home Page

Rate of Neurogenesis

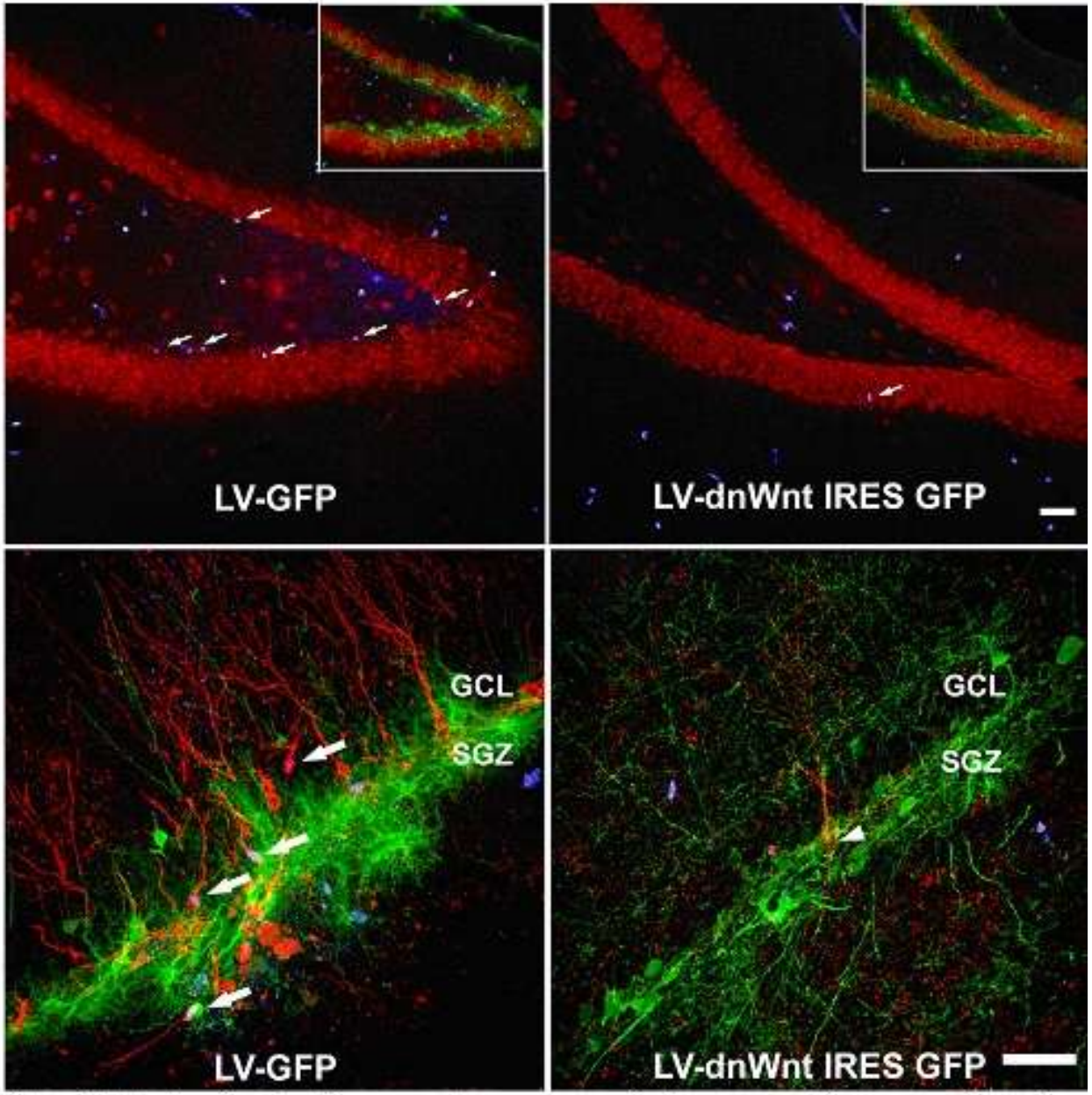


This photo shows dentate gyrus granule cells born in the adult rat over the course of one week. New DNA in dividing cells was labeled with the thymidine analogue, BrdU, injected every other day for one week. Green=BrdU. Red=TUC-4, a marker of immature neurons. Blue=nuclear counterstain.

We have recently found that there are approximately 9400 proliferative cells in the dentate gyrus of young adult rats. These cells divide with a cell cycle time of 25 hours, generating 9000 new cells each day, or more than 250,000 per month. Within one week of their final division, 50% of the newly-generated cells in the adult dentate gyrus can be identified as neurons with the antibody markers TuJ1 and TUC-4. This number of new granule neurons generated each month is 6% of the total size of the granule cell population, and 30-60% of the size of the afferent and efferent populations (stellate cells in the entorhinal cortex and CA3 pyramidal cells). The large number of the adult-generated granule cells supports the idea that these new neurons play an important role in hippocampal function.

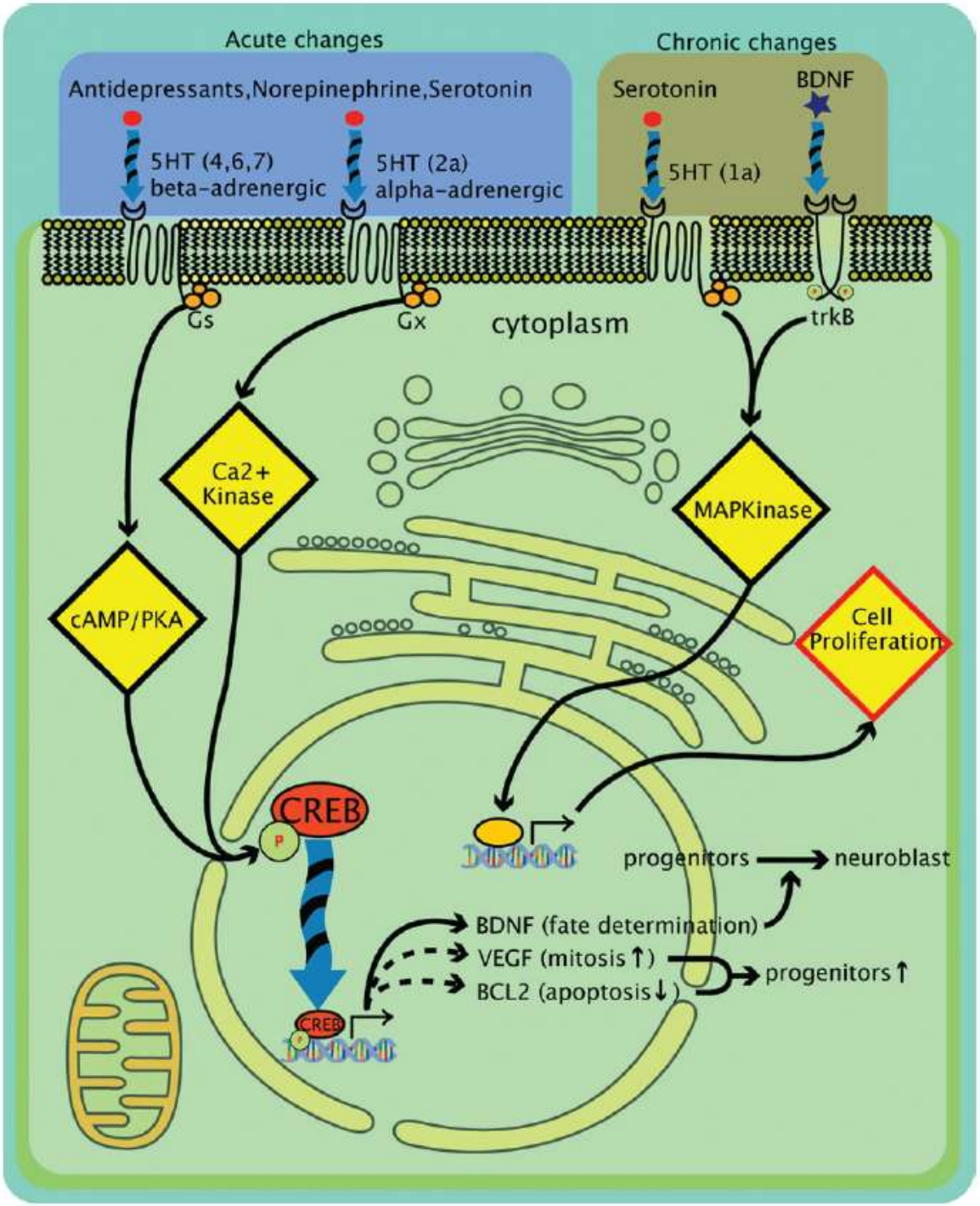
Additional view of dentate gyrus neurogenesis

Additional view of dentate gyrus neurogenesis

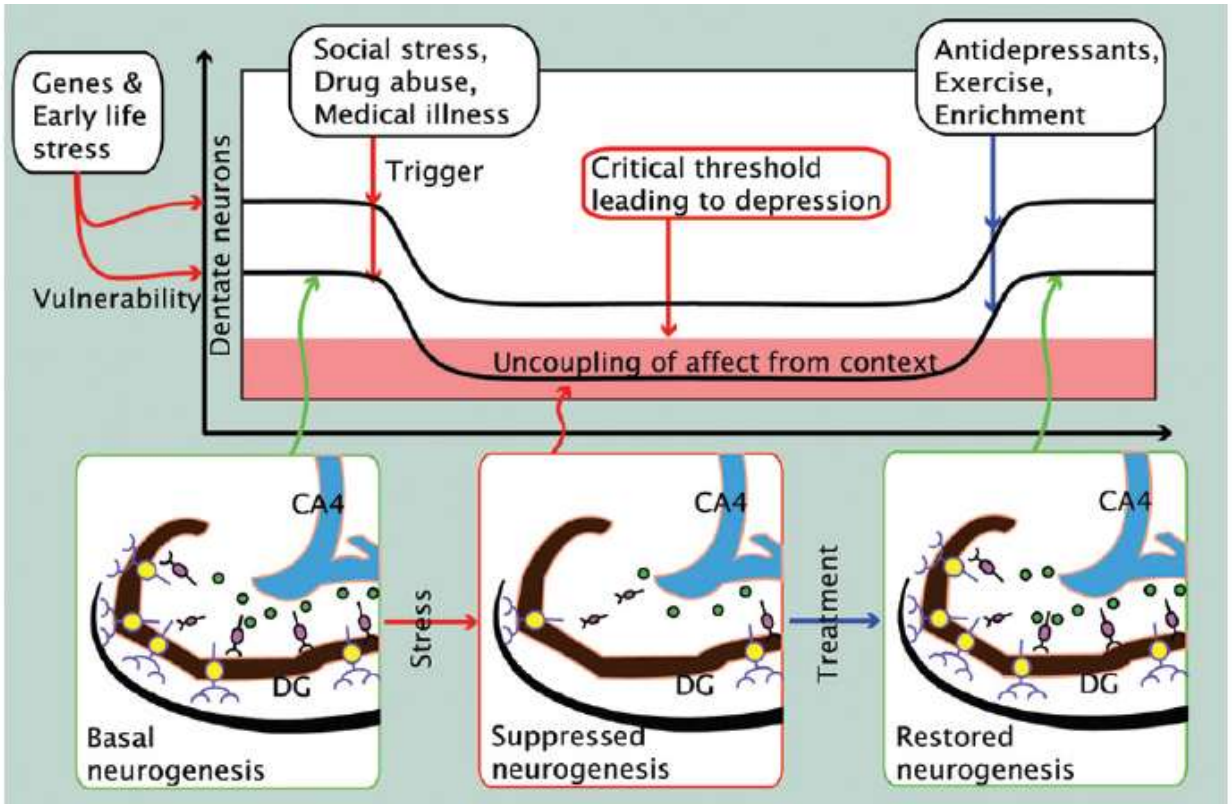


Role of Wnt-signaling in hippocampal neurogenesis. Upper row: Overexpression of a inhibitory form of Wnt (LV-dnWnt IRES GFP, right panel) reduces the number of newborn cells (BrdU in blue) compared to control (LV-GFP, left panel). Lower row: Fewer newborn neurons (DCX in red) are generated following inhibition of Wnt-signaling. For details, please see the article by Lie et al., Nature, 2005.

http://www.helmholtz-muenchen.de/en/idg/group-adult-neurogenesis/research-areas/index.html



How do outside stimuli like drugs or exercise or stress influence neurogenesis?



Function of neurogenesis and neurogenesis theory of depression

What is known about function of neurogenesis

Evidence *supporting* neurogenesis function in response to antidepressants. Impaired contextual fear learning after blockage of neurogenesis, but only with ~4 week delay between neurogenesis block and testing.

Evidence *against* neurogenesis function in response to antidepressants : blocking neurogenesis does not eliminate the beneficial effects of antidepressant therapy or environmental enrichment on measures of anxiety, including novelty induced suppression of feeding (NSF, Science. 2003 Aug 8;301(5634):805-9.PMID: 12907793), light/dark avoidance, elevated plus maze, open field

Function of structures where neurogenesis occurs: dentate gyrus… Responds to (subtle) changes within the same environment (context) but responses do not remap to new environments in the way the hippocampal place cells (pyramidal neurons) do. *"Collectively, these studies demonstrate that neurogenesis is necessary for linking temporally separated events that involve subtle or novel changes within contextually similar settings."* Consequence of dentate gyrus functional integration with CA1/CA3 regions of hippocampus: "novel experiences [encoded by new DG neurons] are linked to episodic memories of similar content [encoded by mature CA1/CA3 neurons]." HYPOTHETICALLY, when DG neurogenesis is impaired, CA1/CA3 pyramidal neurons have to encode the new events, "resulting in impaired segregation of irrelevant, old stimuli from relevant, novel stimuli. Interference with the encoding of novel stimuli impairs the detection of new subtle contextual changes. The inability to detect and appropriately respond to contextual changes results in an "uncoupling" of affect from external context…. The inability to detect subtle changes in external context prevents the recognition [and] eventual reduction or cessation of stress. The negative mood persists, therefore, even after the triggering stress ceases. Unremitting negative mood that is no longer yoked to external context is in essence major depression."

Vol 452|6 March 2008|doi:10.1038/nature06612 nature

Identification of a serotonin/glutamate receptor complex implicated in psychosis

Javier González-Maeso[1,2], Rosalind L. Ang[1], Tony Yuen[1], Pokman Chan[1], Noelia V. Weisstaub[5,6], Juan F. López-Giménez[8], Mingming Zhou[5], Yuuya Okawa[1], Luis F. Callado[9,10], Graeme Milligan[8], Jay A. Gingrich[5,6,7], Marta Filizola[3], J. Javier Meana[9,10] & Stuart C. Sealfon[1,4]

Phenomenon being studied: mechanism of action of hallucinogenic drugs, schizophrenia

Adaptation- (a) sensory gating (b) receptor complexes increase neural signaling repertoire

Behavioral- psychotic behavior in human patients, mouse model of psychotic behavior

Systems- sensory systems

Circuit- DRN serotonergic, and thalamic glutaminergic projection to somatosensory cortex

Cell biological (including electrophysiological)- complexing of distinct membrane bound metabotropic receptor types. Many cell biological experiments: physiological responses to agonist, colocalization (BRET, FRET), receptor binding affinity assays. 2AR must be complexed with the GLUR2 to allow 2AR agonist to activate Gαi1,2,3

Genetic- GLUR2 sequence required for transmembrane domain mediating 2AR complexing

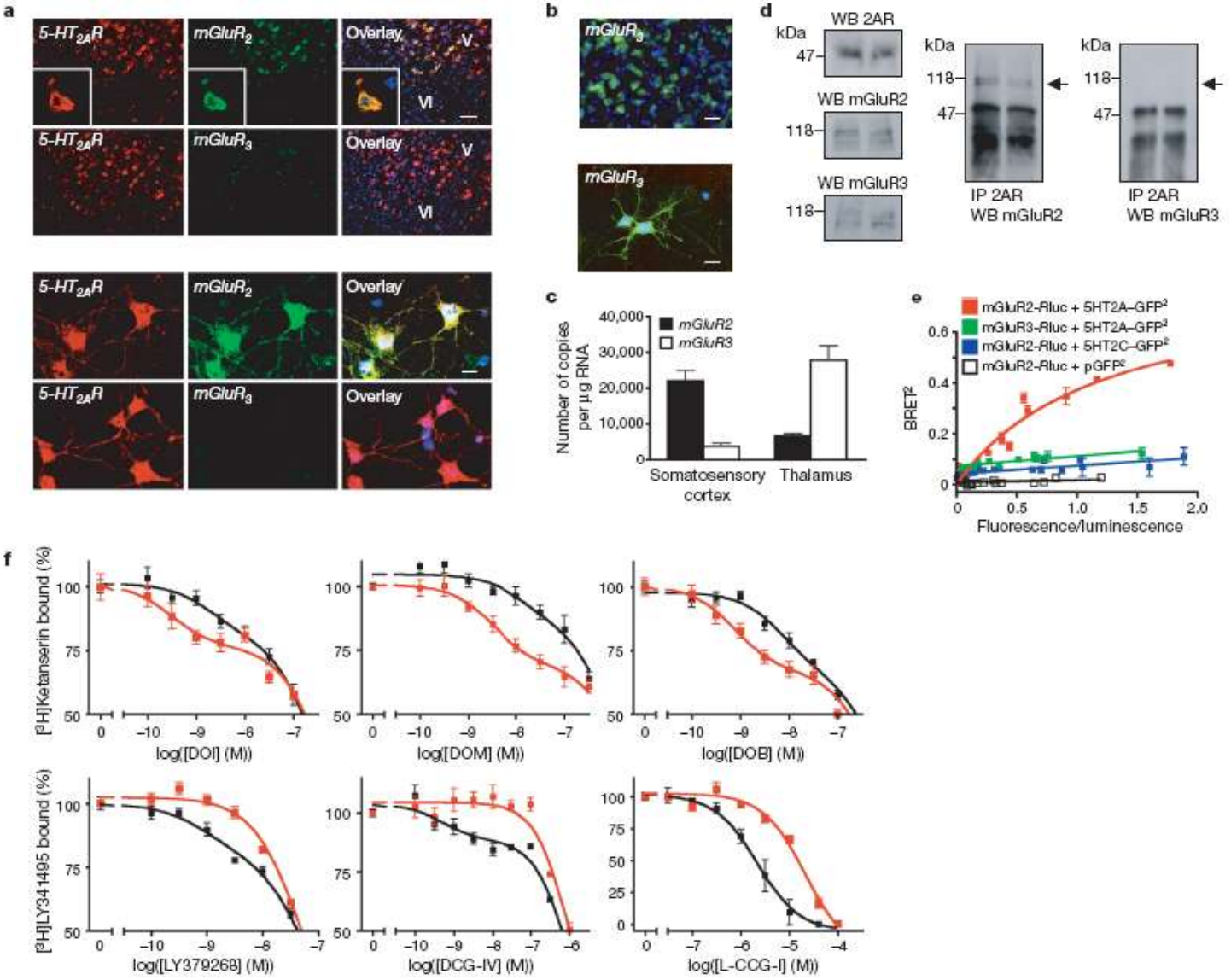


Figure 1 | 2AR and mGluR2 co-localize and interact. **a**, *2AR* and *mGluR2*, but not *mGluR3*, are co-expressed in cortical neurons. Top, mouse somatosensory cortex; bottom, mouse cortical primary culture. Scale bars, 50 µm (top) and 10 µm (bottom). Nuclei are blue. Inset: co-expressing neuron. **b**, FISH for *mGluR3* in thalamus. Top, mouse thalamus; bottom, thalamic primary culture. Scale bars, 25 µm (top) and 10 µm (bottom). **c**, mRNA levels measured by real-time PCR (Error bars show s.e.m.; $n = 6$ per group). **d**, Specific co-immunoprecipitation of 2AR and mGluR2 in duplicate human frontal cortex samples (arrows). WB, western blot; IP, immunoprecipitation. **e**, BRET2 shows specific 2AR and mGluR2 interaction in HEK-293 cells. Data are means ± s.e.m. ($n = 3$). The mGluR2/2AR curve is fitted better by a saturation curve than by a linear regression, F test ($P < 0.001$). The other co-transfection data sets show linear regressions. **f**, Top, [^{3}H]ketanserin displacement curves in mouse SCx membranes. 2AR agonist affinities were higher in the presence of the mGluR2/3 agonist LY379268 at 10 µM (red) than in vehicle alone (black). [^{3}H]LY341495 displacement curves (bottom panels). mGluR2/3 agonist affinities were lower in the presence of the 2AR agonist DOI at 10 µM (red) than in vehicle alone (black). DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)-glycine; L-CCG-I, (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)-glycine.

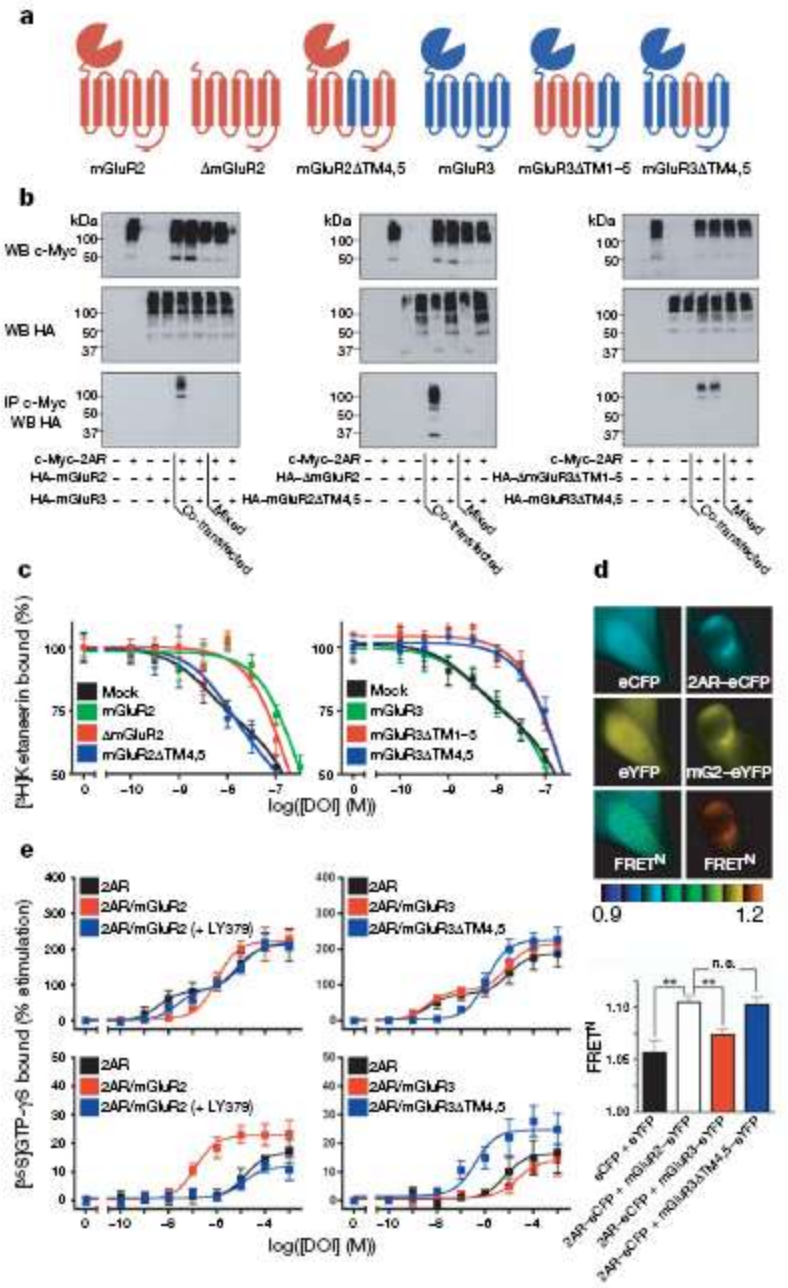
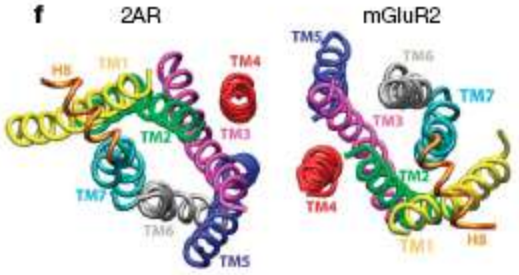


Figure 2 | mGluR2 transmembrane domains 4/5 mediate association with 2AR. **a**, mGluR2/mGluR3 chimaeras studied. **b**, c-Myc–2AR and haemagglutinin (HA)–mGluR2/mGluR3 chimaera co-immunoprecipitations. Cells separately expressing each construct were also mixed. WB, western blot; IP, immunoprecipitation. **c**, 2AR competition binding in cells stably expressing the 2AR and transfected with mGluR2/mGluR3 chimaeras. Error bars show s.e.m. ($n = 4$). **d**, FRET in cells expressing 2AR tagged with enhanced cyan fluorescent protein (eCFP) and either mGluR2, mGluR3 or mGluR3ΔTM4,5 chimaera, all tagged with enhanced yellow fluorescent protein (eYFP). Pseudocolour images represent normalized values ($FRET^N$). Numbers of samples: eCFP + eYFP, $n = 19$; 2AR–eCFP + mGluR2–eYFP, $n = 43$; 2AR–eCFP + mGluR3–eYFP, $n = 31$; 2AR–eCFP + mGluR3ΔTM4,5–eYFP, $n = 27$. Two asterisks, $P < 0.01$; analysis of variance with Dunnett's post hoc test. n.s., not significant. Error bars show s.e.m. **e**, DOI-stimulated [^{35}S]GTP-γS binding in membranes followed by immunoprecipitation with anti-$G\alpha_{q/11}$ (top) or anti-$G\alpha_{i1,2,3}$ (bottom). Cells stably expressing 2AR were transfected with mGluR2, mGluR3 or mGluR3ΔTM4,5. The potency of DOI activating $G\alpha_{i1,2,3}$ was significantly increased when the 2AR was co-expressed with either mGluR2 or mGluR3ΔTM4,5, an effect abolished by 10 μM LY379268 (LY379) ($P < 0.001$ by F test). Data are means ± s.e.m. for three experiments performed in triplicate. **f**, Ribbon backbone representation of the transmembrane helices of the 2AR–mGluR2 heteromer model, seen from the intracellular face.



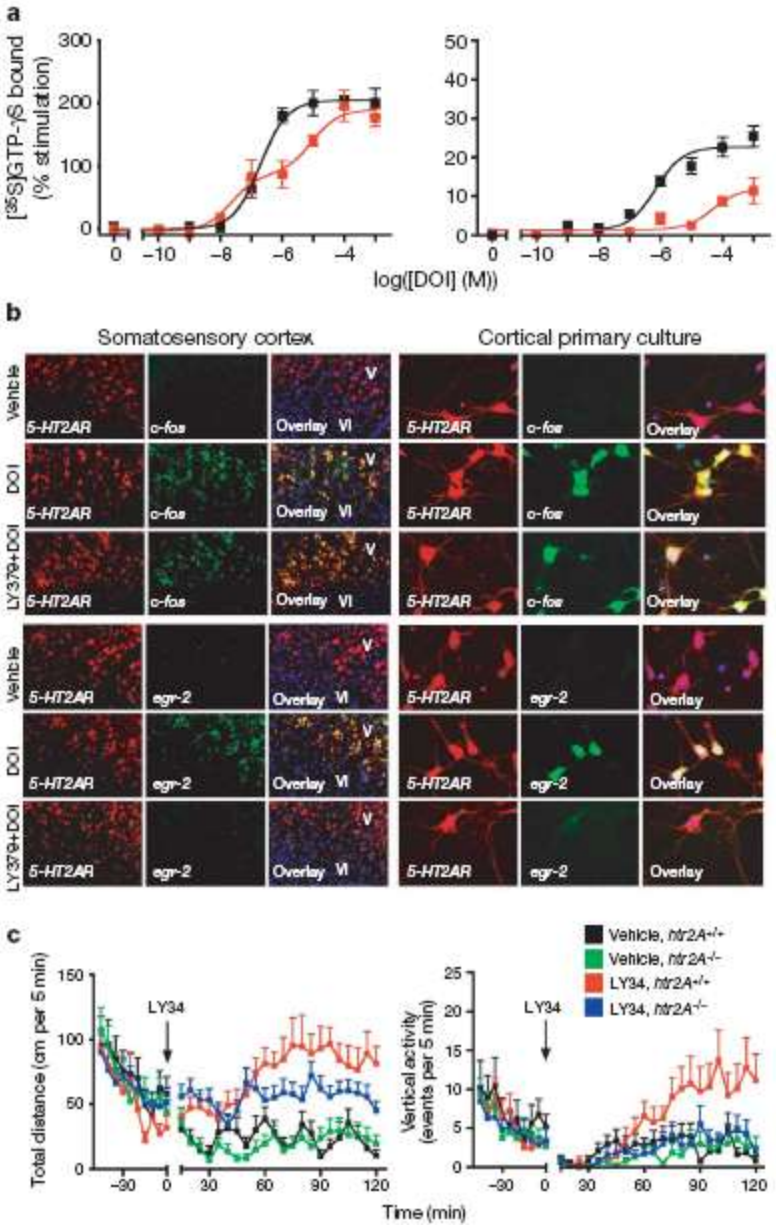


Figure 3 | 2AR–mGluR2 complex-dependent modulation of cellular and behavioural responses. **a**, DOI-stimulated [^{35}S]GTP-γS binding in primary culture membranes followed by immunoprecipitation with anti-G$\alpha_{q/11}$ antibodies (left) or anti-G$\alpha_{i1,2,3}$ antibodies (right). DOI G$\alpha_{i1,2,3}$ activation potency was significantly decreased by 10 μM LY379268 (red) compared with vehicle alone (black). Data are mean ± s.e.m. for three experiments performed in triplicate. **b**, FISH in mice injected with vehicle or 2 mg kg^{-1} DOI 15 min after injection with vehicle or 15 mg kg^{-1} LY379268 (left), and in primary cultures treated with 10 μM DOI 15 min after being pretreated with vehicle or 10 μM LY379268 (right). Nuclei are blue. Scale bars, 50 μm (left) and 10 μm (right). **c**, Distance and vertical activity induced in *htr2A*$^{+/+}$ and *htr2A*$^{-/-}$ mice by the mGluR2/3 antagonist LY341495 (LY34) at 6 mg kg^{-1}. In *htr2A*$^{-/-}$ mice, the effect of LY341495 on distance was decreased ($P < 0.05$; Bonferroni's post hoc test of two-factor analysis of variance), and its effect on vertical activity was absent ($n = 30$–32). Error bars show s.e.m.

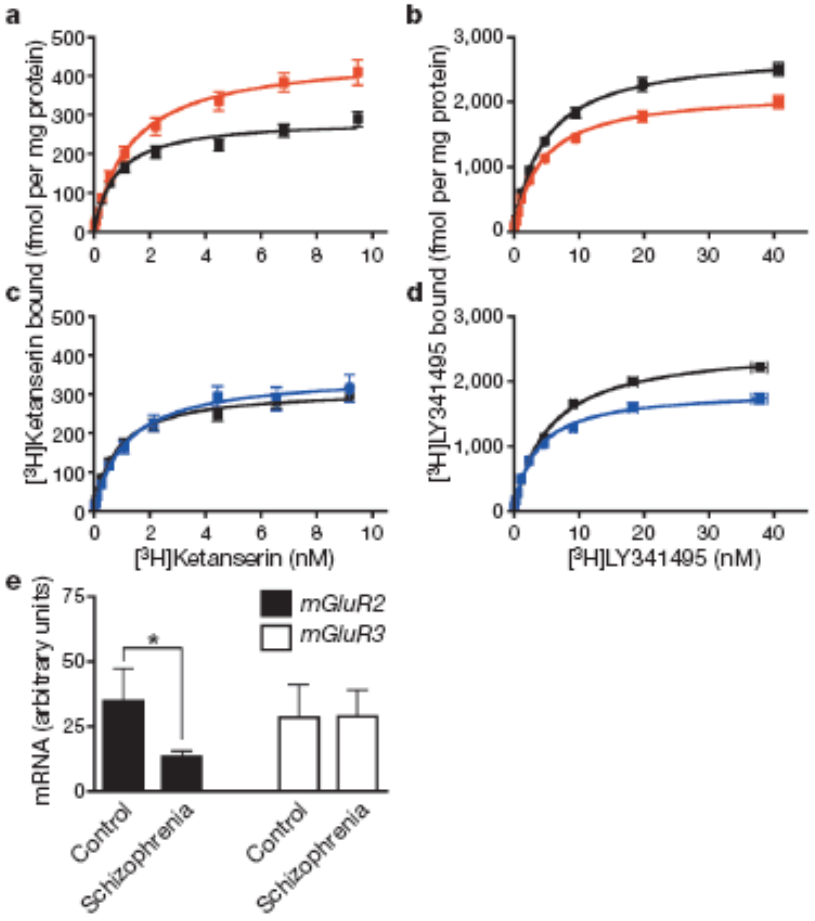


Figure 4 | 2AR is increased and mGluR2 is decreased in schizophrenia. **a**, **b**, Frontal cortex membrane receptor binding assays from untreated schizophrenic subjects (red; $n = 13$) and matched control subjects (black; $n = 13$). In schizophrenia, [^{3}H]ketanserin binding (**a**) was higher and [^{3}H]LY341495 binding (**b**) was lower ($P < 0.05$; Student's t-test). Error bars show s.e.m. **c**, **d**, Receptor binding in antipsychotic-treated schizophrenic subjects (blue; $n = 12$) and matched control subjects (black; $n = 12$). In treated schizophrenia, [^{3}H]ketanserin binding (**c**) was unaffected and [^{3}H]LY341495 binding (**d**) was lower ($P < 0.05$). Error bars show s.e.m. **e**, *mGluR2* mRNA expression is decreased in untreated schizophrenic subjects ($n = 7$) compared with matched control subjects ($n = 7$; asterisk, $P < 0.05$; error bars show s.e.m.).