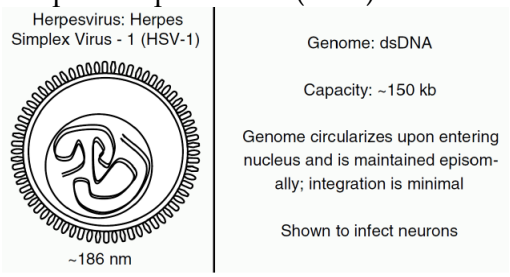
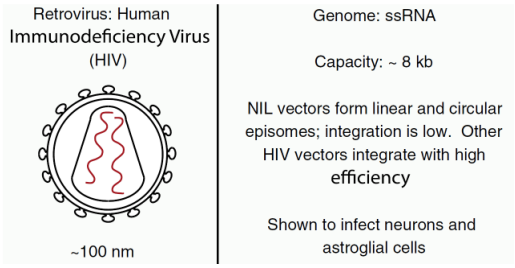
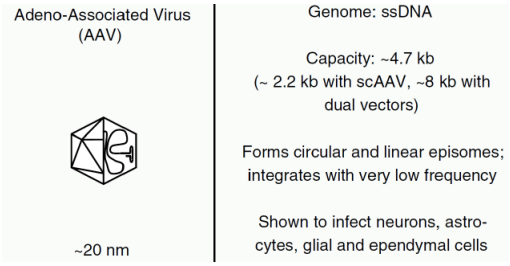
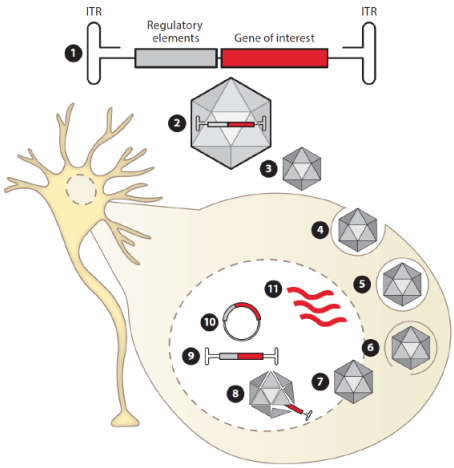
Recombinant viral vectors for gene delivery (Michel van der Oeven)

What is the difference between transduction and transfection?	Infection/Transduction - Introduction of genetic material with virus Transfection - Introduction of genetic material without virus (methods to increase permeability of the cell) • Cannot be used in vivo
What is the advantage of using viral vectors compared to other strategies of delivery?	Why viral vectors? • Simple and small biological agent • Stable transport - Capsid of virus protects the genetic material • Makes use of host cell protein machinery

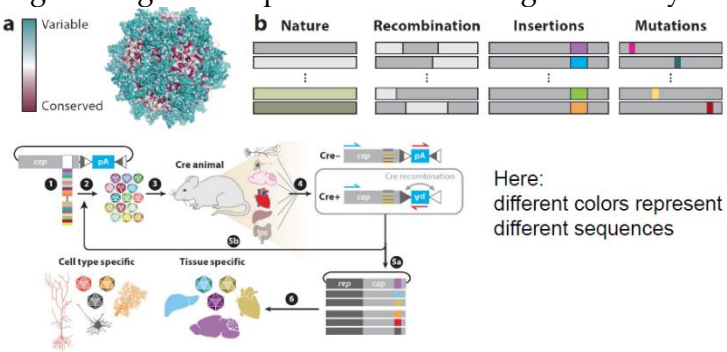
Which factor should you consider when choosing a viral vector?	Factors to consider:
	• Transduction efficiency of target cells (tropism of virus)
	• Cloning capacity (gene must fit the viral genome)
	• Antero/retrograde capacities
	• Chromosomal or episomal expression
	  ○ Might introduce random gene interferences
	  ○ Especially problematic for cells that divide
	• Toxicity

What are the main advantages and disadvantages of Herpes virus?	Herpes Simplex Virus (HSV)  Advantages: • Large cloning capacity - 150 kb • Remains episomal • Naturally neurotrophic • Short incubation time (days) - double stranded virus, makes duplication process faster • Made replication deficient (in order not to infect the entire brain) Disavantages: • Limited infection of glia cells • Short duration of expression (1-4 weeks) • Presence of helper virus is necessary for HSV to replicate
What are the main advantages and	Retrovirus (e.g. Lentivirus)

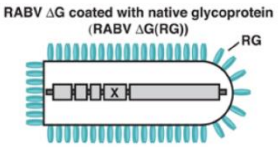
disadvantages of retrovirus?	 Advantages • Derived from human immunodeficiency virus • Pseudotyping (changing the capsid proteins) allows for target specificity • Large cloning capicity ~8 kb • Long expression of transgene - 3 months Disadvantages • Very small area of infection
What are the main advantages and disadvantages of adeno-associated virus?	Adeno-associated virus  Advantages • Episomal • Good diffusion in brain tissue • Low immune response, no side effects • Long (permanent?) transgene expression • Good safety level - When infecting human/primate cells, leads to very low immune response Disadvantages • Smaller cloning capacity ~5kb • Longer incubation period • Presence of helper virus is necessary for packaging of virus
What is the overall AVV genome structure?	AAV genome structure

	 • 2 inverted terminal repeats • Viral genes are replaced by transgene • Then a helper virus is necessary for packaging

Which factors define cell and region specifity in viral vectors?	Factors that determine cell-type and region specificity
	• Serotype: Capsid proteins
	  ◦ Serotype 9 (N-linked galactose) - Crosses the blood brain barrier
	• Route of delivery
	  ◦ Intracranial/Stereotaxic surgery
	  ◦ Intra-CSF - Cerebral spinal fluid
	  ◦ Systemic - Virus need to cross the BBB
	• Viral titer - Number of copies of viral vectors
	• Injection volume
	• Flow rate and direction of injection
	• Gene regulatory elements
	  ◦ Strong cell specific promoters - e.g. CaMKII (only works with strong promoters)
	  ◦ Weak cell-specific promoter - Needs another system (cre recombinase);
	  • Weak promoter drives transcription of cre
	  • Cre is then permanently expressed

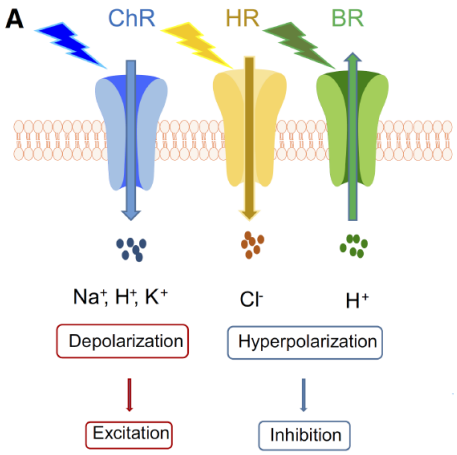
Which techniques can be used to engineer AAV capsids?	Engineering AAV capsids to increase target diversity  • Importance of systemic delivery: Less invasive surgery

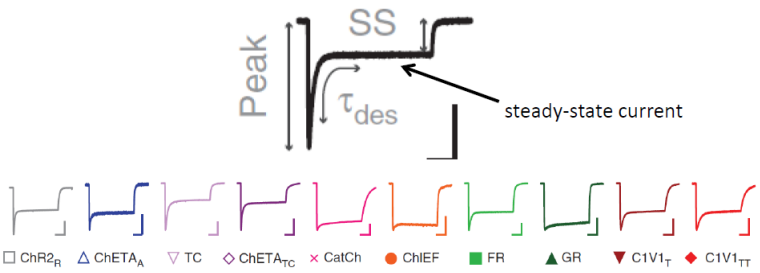
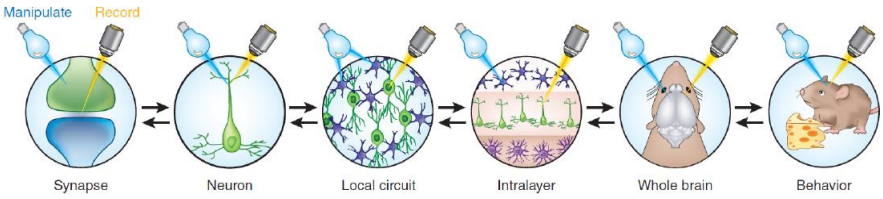
	• Changing capsid properties of AAV
	
	• Consideration for systemic delivery:
	  ○ More virus required
	  ○ More immune response
	  ○ Gene regulatory elements specific for the nervous system
	• Transynaptic tracing - Virus jumps retrogradely only once

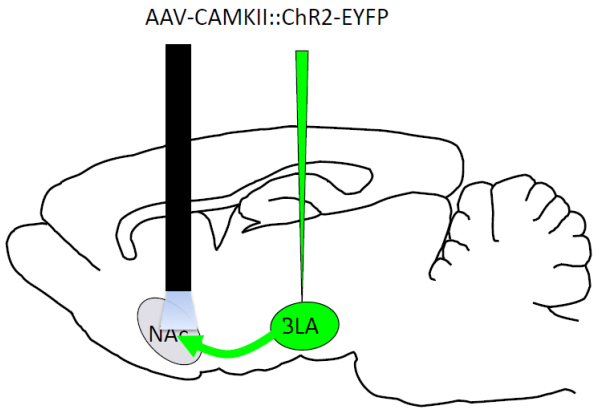
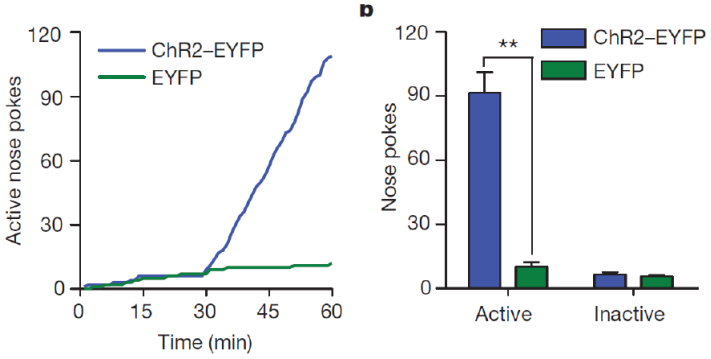
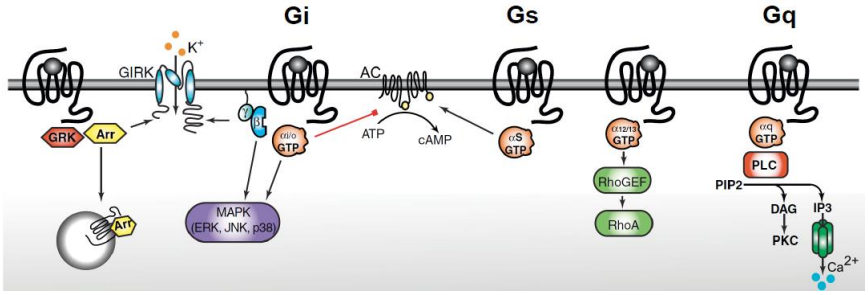
What are the main advantages and disadvantages of rabies virus?	Rabies virus
	 
	
	• Cloning capacity ~5 kb
	• Naturally neurotrophic
	• Selective retrograde transsynaptic tracing
	  ○ Depends on the expression of RABV-G in starter cells
	  ○ RV pseudotyped with EnvA - Only infects cells that express the TVA receptor (TVA and RABV-G can be expressed in starter cells in a cre-dependent manner)
	Disadvantages:
	• Neurotoxic - Cells die in 1-2 weeks
	• Not suitable for behavioral experiments
	• Safety restrictions (DMII level)
	• Potential bias in transsynaptic transversal efficiency in different cell-types

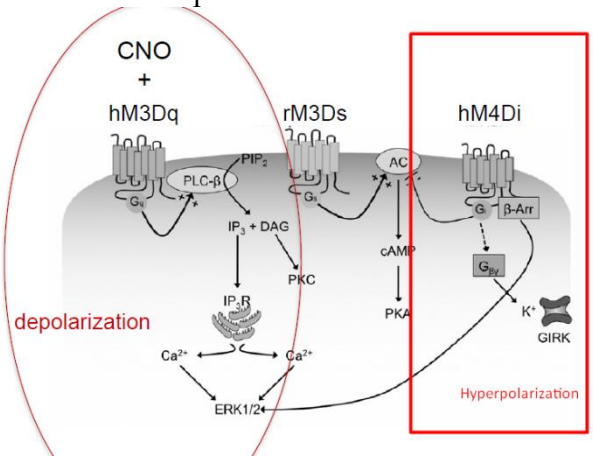
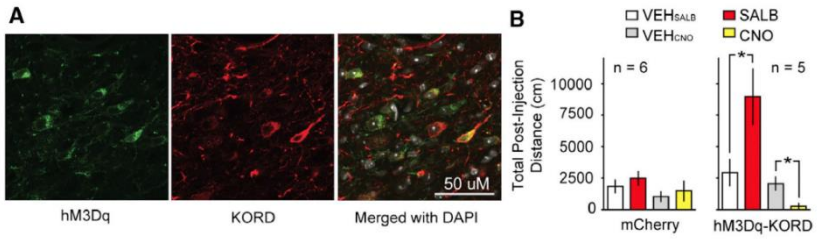
Opto- Chemogenetics (Michel van der Oeven)

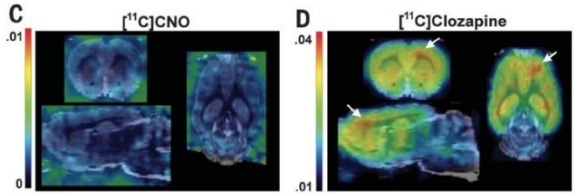
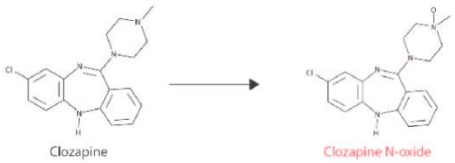
How does opto and chemogenetics compare to other brain manipulation techniques?	Relevance of opto and chemogenetics
	• Brain is high complex, trillions of connections, thousands of cell types
	• Lesion:
	  ○ Low temporal resolution, no cellular specificity, only loss of funtion
	• Pharmacology:
	  ○ Low temporal resolution (hours), cellular specificity limited, loss and gain of function can be studied
	• Electrical stimulation:
	  ○ High temporal resolution, not cell-specific, only gain of function

What are the types of opsins currently available?	Light sensitive proteins (opsins) • Micro-organisms use them for energy metabolism  • Channelrhodopsin - Stimulated by blue light, depolarizes the cell • Halorhodopsin - Stimulation by yellow light, hyperpolarizes the cell • Archeorhodopsin - Stimulation by green light, hyperpolarizes the cell
Which factors influence the function of opsins?	Factors that influence the function of opsin • Wavelength • Intensity of illumation • Duration of ilumination • Channel kinetics (molecular structure can be modified)

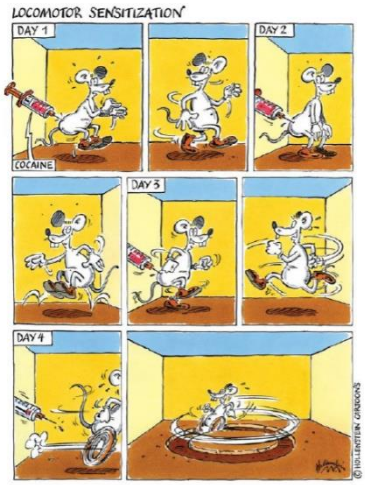
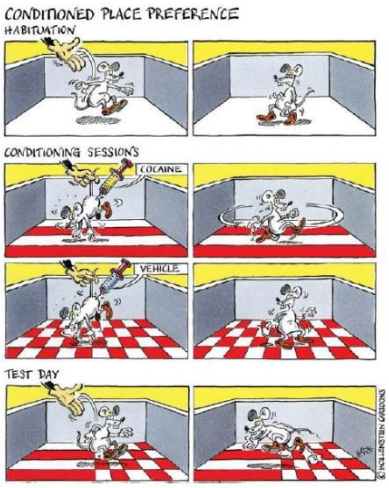
	 • Expression level • Must match the dynamics of the cell type in which it is being expressed
How can opsins be expressed in mammals?	Expression in mammalian cells • Transfection (ex vivo only) • Viral vectors • Transgenic mice
How can the light be delivered in an opto setup?	Delivery of light • Optic fiber • Cannula • Laser/LED - LED is a more recent development (wireless is possible) ○ Heat considerations - Inhibiting neurons causes more tissue heat (more time)
What are the levels of observations that can be done to assess the effect of optogenetic manipulations?	Read-out modalities  • Two photon is needed to observe neurons in vivo • Physiology - in vivo and ex vivo (patching for real time; Fos for post-hoc analysis) • Behavior
How can optogenetics be used to study motivation?	Modulating projection • Expression in BLA, projecting to Nac

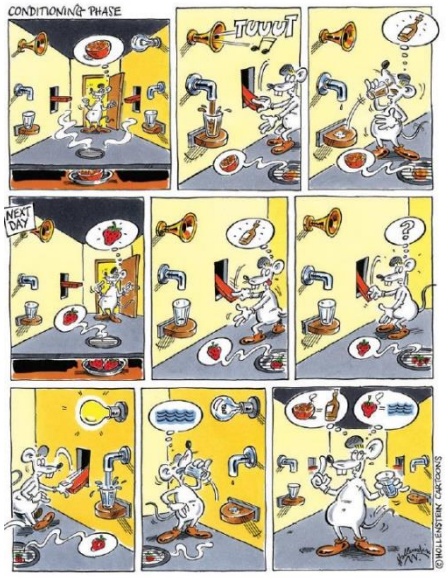
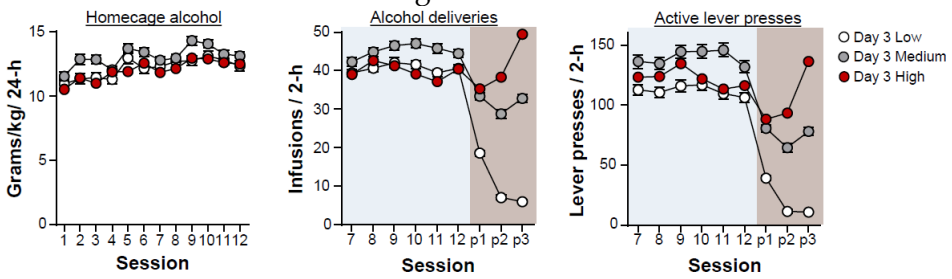
	•  • Skinner box - Reward = activation of neurons  ○ Evidence that BLA-Nac mediates reward learning
Which new technologies developed on the concept of light-sensitive ion channels?	Expanding toolbox • Light sensitive G-protein coupled receptor • Light sensitive proteins
What is a DREADD? How do they act in the cell?	DREADDs - Designer Receptor Exclusively Actived by Designer Drugs • GPCRs which are activated by inert small molecules ○ One third of drugs act on GPCRs - they are often expressed in multiple cell types and the drugs act on multiple types of GPCRs
What is a GPCR? What are the types of GPCR and how do they function in the cell?	GPCR - 7 transmembrane, C-terminal inside the cell  ○ Active G-protein phosphorylates targets downstream ○ Beta-arrestin - Signals endocytosis of the receptor

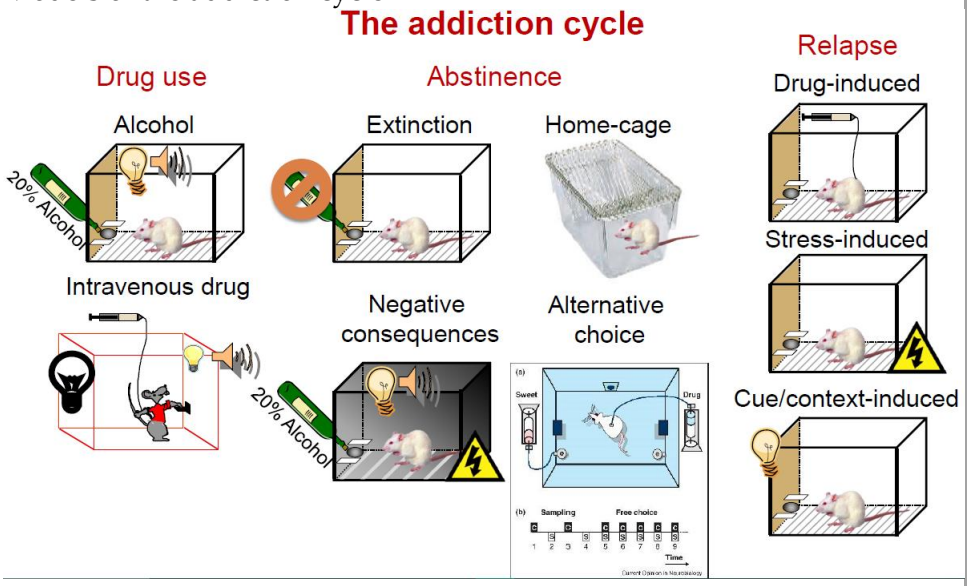
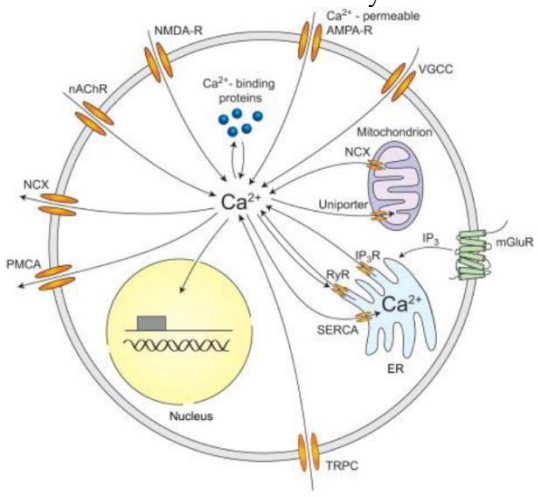
	Types of GPCRs ○ Gi - Inhibitory (decreases adenylyl cyclase, activates potassium channel, hyperpolarizes cells) ○ Gs - Stimulating; usually not used ○ Gq - Stimulating; Activation of PKC and release of calcium
Which factors need to be taken into account when choosing a DREADD?	Factors of DREADD • Ligand should not bind endogenous receptors • Receptor should not bind to endogenous proteins • Constitutive activity - Receptor becomes active without a ligand • Expression levels • Desensitization ○ Opioid tolerance - Mediated by uncoupling of signal pathways (compensatory upregulation of cAMP), not by receptor endocytosis • Canonical and non-canonical pathways
What are the two main types of muscarinic DREADDs?	Muscarinic receptor DREADDs 
What is the advantage of using opioid DREADDs? Why is their application severely limited?	KORD-DREADD - Insensitive to endogenous opioids, sensitive to molecules derived by Salvia (Salvinorin B)  • E.g. Expression in GABAergic neurons -> Expression of different DREADDs in different cell types 

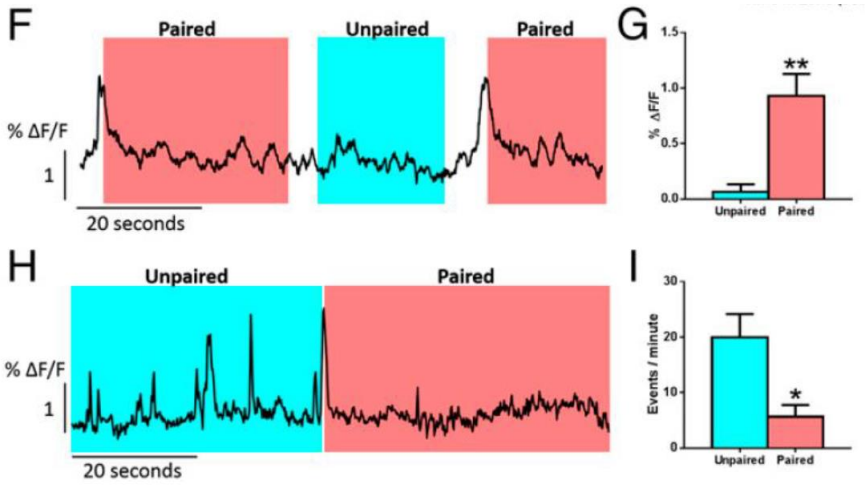
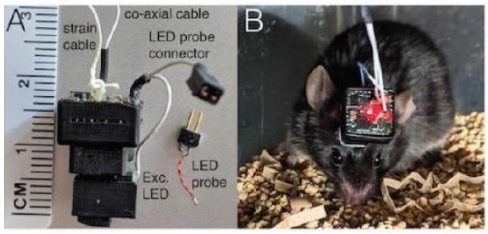
	• Main disadvantage - 100% DMSO is required to dissolve
What is the main problem with using CNO?	Issue with CNO • CNO is metabolized in the liver into clozapine -> many effects; clozapine binds more effectively to DREADDS + many other receptors • CNO does not cross the BBB -> clozapine does  ○  • If you wait long enough (2-3h), control group without DREADDs also present changes in behavior • Conclusion: CNO can be used with the right control (careful with multiple injections over multiple days); control for DREADD expression and CNO injection
Which is more clinically relevant: opto or chemogenetics?	Chemogenetics are more clinically relevant than optogenetics • Less invasive

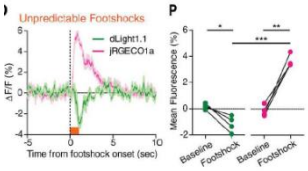
Addiction/Calcium imaging (Nathan Marchant)

What is drug addiction?	Drug addiction • DSM-V definition: Use for longer periods of time, wanting to reduce use while being unsuccessful to do so; tolerance/withdraw • Stages of addiction: Safe use; excessive use; compulsive use; abstinence; relapse
What are the models of addiction for loss of control?	Models of addiction • Locomotor sensitization - Increase in locomotor response after repeated exposure  • Conditioned place preference - Rats prefer the chamber in which they had received a drug  • Advantages: Simple, high throughput, easily reproducible • Disadvantages: Too simple
What are the models of addiction for self-	Models: Operant self-administration

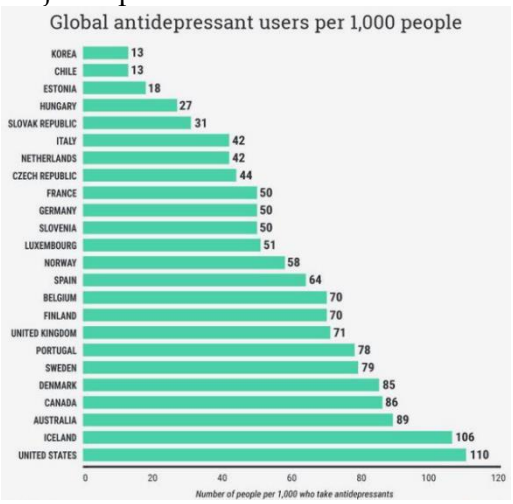
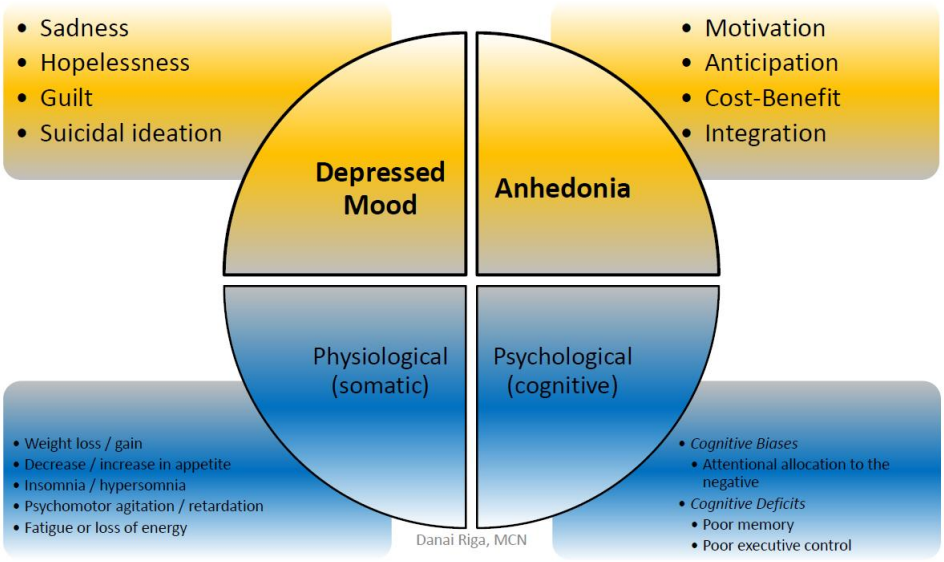
administration ?	 • Escalation model: Training for 6 hours a day leads to increase of intake
What are the models of addiction for compulsive use?	Compulsive use: seeking and taking drugs despite obvious deleterious effects • Model: Associate drug use with foot shock • Three-criteria model of drug addiction: ○ Persistent drug seeking when unavailable ○ Resistance to punishment ○ Increased motivation (assessed by progressive ratio) • Extinction (drug not available); Punishment (shock); Progressive ratio (increasing number of lever presses required to receive the drug)
Why not all individuals that take addictive drugs become addicted?	Not all individuals that take drugs become addicted  • Difference in punishment learning/3 criteria model
What are models of addiction to assess relapse?	Relapse: re-exposure to environment contexts associated with drug use leads to relapse • Model: Test for drug seeking behavior, not drug taking ○ Stress-induced reinstatement - Rats that receive foot shocks do more lever presses ○ Cue-induced reinstatement - Memory of the light triggers the memory of the drug

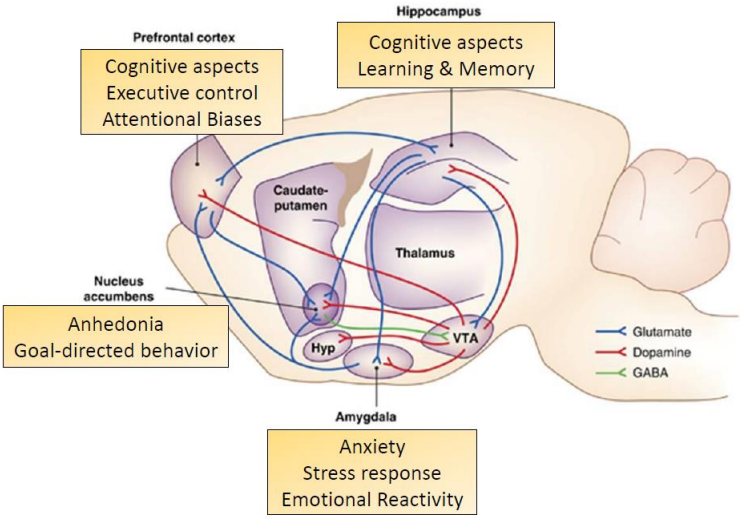
What are the main limitations of animal models of addiction?	Models of the addiction cycle  • Limitations of extinction-based relapse models: extinction does not model human abstinence ○ Alternative: experimenter-imposed or self-imposed abstinence • Ecological limitation: What choice does a rat have but to press a lever?
What is the 'alternative choice' model?	Exclusive choice: drugs or food? • Most rats choose food over drugs when the choice is exclusive • This even works for social interaction over drugs ○ Social reward is higher in the value ladder over drugs
What is the biological relevance of calcium imaging?	In-vivo calcium imaging • Fiber photometry/Miniscope • Why calcium: Universal signal in excitable cells; High variation between baseline and activity • 

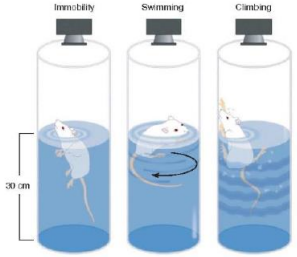
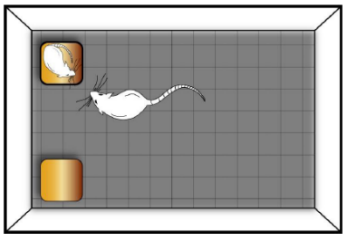
What is GCaMP?	GCaMP - Invented in 2001 (Nakai et al) • GFP+calmodulin+M13 • Needs 4 calcium ions to allow for the conformation change • GCaMPs are not great for neurons that spike very fast and constantly (high baseline signal) • Limitation: variations of expression in different sub-populations of neurons
What happens to the NAc when a rat enters the environment in which it received cocaine before?	Applications for addiction • Nucleus accumbens - Big spike of activity when the rat enter the cocaine environment 
What are miniscopes?	Miniscopes - Invented after two-photon microscopes became commonplace  • NINscope - deep brain recording
What are the advantages and disadvantages of fiber photometry vs miniscopes?	Fiber photometry Advantages: • Restricted expression of GCaMP • Data analysis is simple Disadvantages: • No cellular resolution Miniscopes Advantages: • Single-cell resolution Disadvantages: • Technically very difficult (3/100 rats)

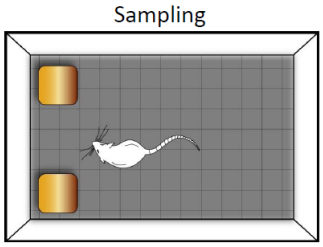
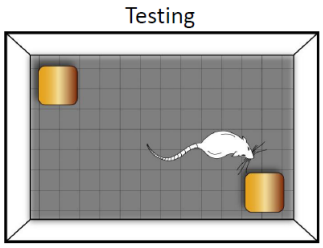
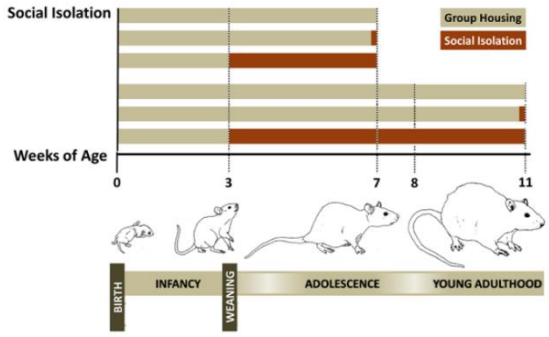
What is the future of fiber photometry and miniscopes?	Intensity-based genetically encoded dopamine receptors • Can be used in conjunction with GCaMP • 

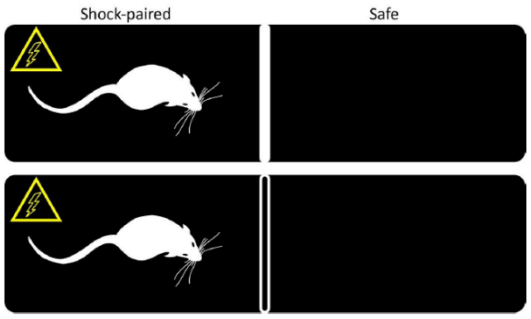
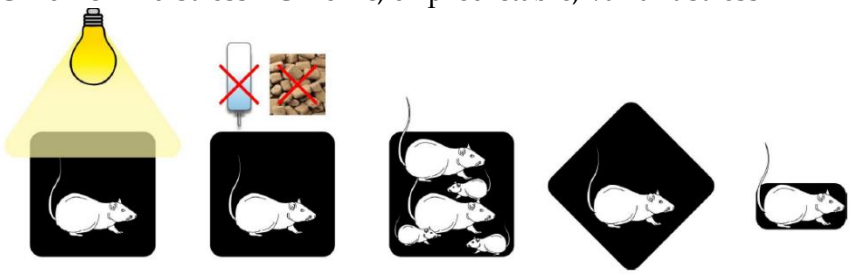
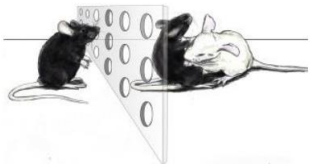
Defeating depression: preclinical models (Danai Riga)

What is the prevalence of depression in the world?	Major depressive disorder:  • Leading cause of disability - 5% of the world population, 7% of europeans, 10% of brazilians • 30-40% genetic heritability
What is the problem with the current treatment for depression?	Treatments for MDD were discovered almost 70 years • TCA • SSRI • MAOI • 65% of patients that take the drug still present symptoms; 20% are totally irresponsive to treatment
What is the diagnostic criteria for depression?	Diagnostic criteria 

What is the implicated circuitry in depression?	Implicated circuitry  • Limbic system - Anhedonia • Amygdala - Anxiety, stress response, emotional reactivity • PFC - Cognitive deficits • Hippocampus - Memory deficits

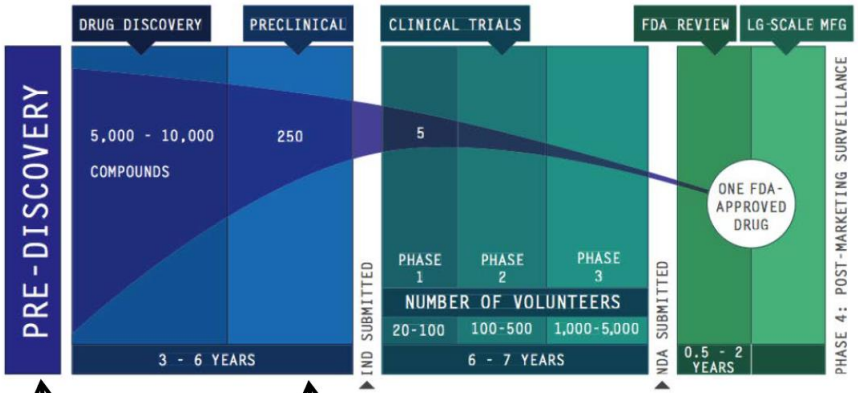
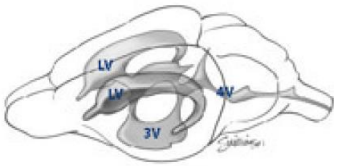
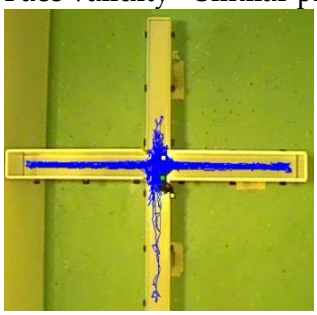
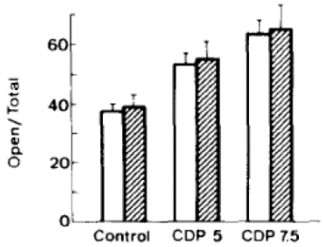
Exam question: What are the types of validity criteria for depression models?	Validity criteria
	• Etiology: similar causes
	  ○ Stress
	  ○ Genetic make-up - Tryptophan hydroxylase, serotonin transporter
	• Construct validity
	  ○ Hippocampal anatomy
	• Face validity
	  ○ Hopelessness/ behavior despair -> forced swimming test
	 
	
	  ○ Anhedonia -> Social approach avoidance, instrumental responding to reward (too high or too low)
	 
	  ○ Cognitive symptoms - Novel object recognition, social recognition

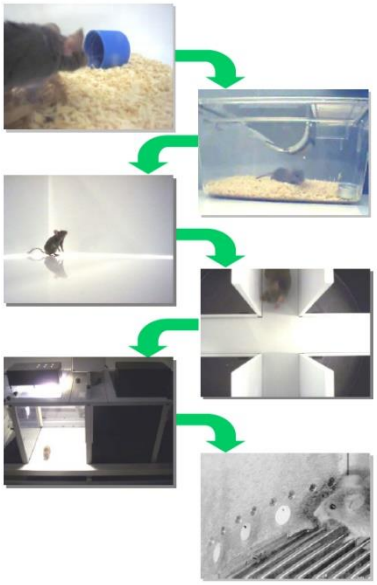
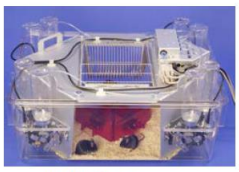
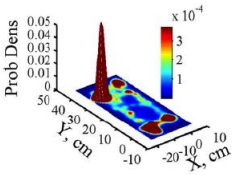
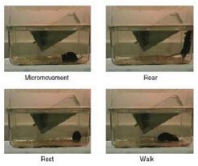
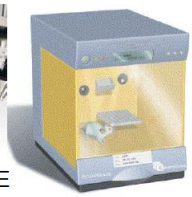
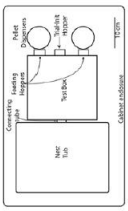
	  ○ Memory -> Object place recognition, water maze 
How could you induce depression-like symptoms in rodents?	Preclinical models of depression • Maternal deprivation - Allow only 1 hour a day for feeding  ○ Depressive-like states during adulthood - Less sucrose preference, less time suspending from the rod in tail suspension test • Juvenile isolation - Deprivation of contact during critical periods (21-28)  ○ Reduced excitability of the Ralphe nuclei ○ Reduced sucrose preference • Learned helplessness - Consecutive footshocks in one context

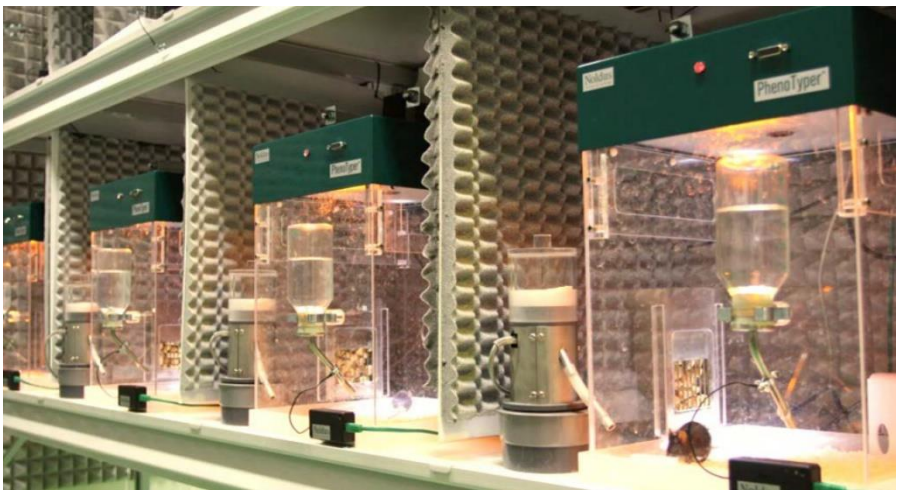
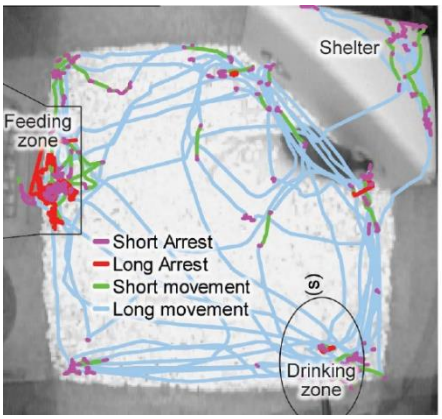
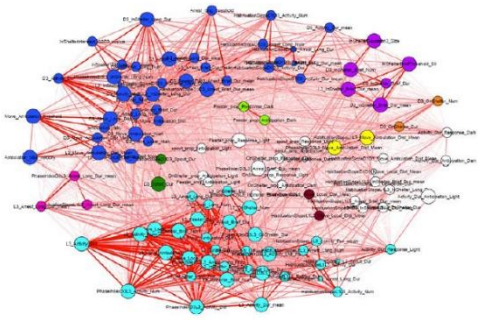
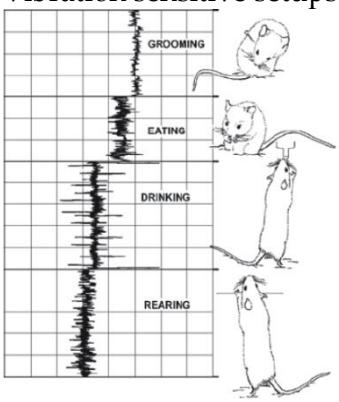
	 ○ Division of individual mice into LH-susceptible and not susceptible • Chronic mild stress - Chronic, unpredictable, variant stress  • Social defeat - Resident-intruder paradigm; only work with males (social hierarchy paradigm) 
What is the usefullness of Social defeat-induced persitence stress over other depression models?	Social defeat-induced persistence stress  • 5 episodes in 5 days - Generates defeat and subordination • Then the animal is put into social isolation in a empoverished environment ○ Results in compulsive behavior (sucrose seeking), worsening of spatial and social memory ○ There is a group of rats susceptible and not susceptible to SDPS ○ Increase in alcohol seeking ○ SDPS increases expression of extracellular matrix -> reduces inhibitory transmission in the hippocampus • ChABC degrades extracellular matrix, recovers phenotype
	Comparative table:

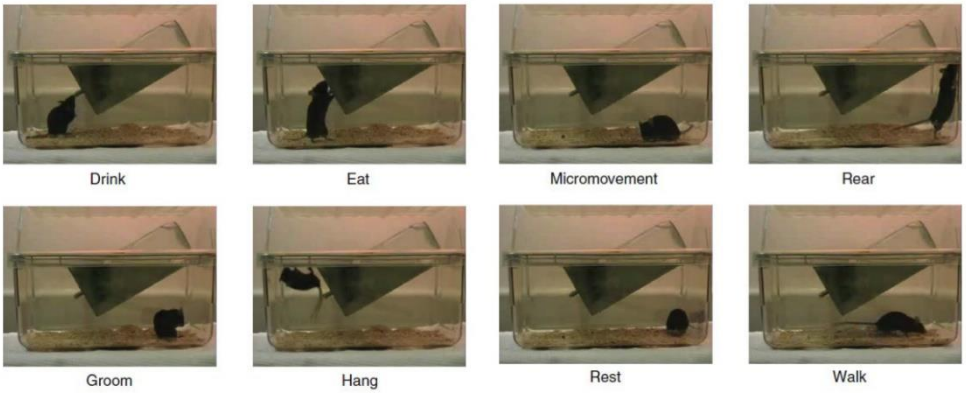
Animal model	Face Validity	Etiological Validity	Construct Validity	Predictive Validity	Limitations
Maternal Deprivation	Behavioral despair Anhedonia Cognitive decline Gender Dichotomy	Good	Hippocampus HPA axis	Antidepressant response	Vulnerability to develop depression rather than depressive state
Early-life isolation	Behavioral despair Anhedonia Cognitive Decline	Good	Hippocampus	Environmental Enrichment	Vulnerability to develop depression rather than depressive state
Learned Helplessness	Behavioral despair Anhedonia Cognitive decline	Poor (non-naturalistic) BUT –congenital effects	Hippocampus HPA axis	Antidepressant response	Technical replicability
Chronic Mild Stress	Behavioral despair Anhedonia Cognitive decline	Poor (non-naturalistic)	Hippocampus HPA axis	Antidepressant response	Antidepressant administration & depression evaluation during/ acutely after stress
Social defeat	Anhedonia Cognitive decline Addiction vulnerability	Good Genetic susceptibility	Hippocampus HPA axis	Antidepressant response	Difficult to implement in female population

Home cage based phenotyping (Maarten Loos)

When are animals used in drug development?	Animal models in drug development  • Pre-discovery - Understanding biology of the disease • Pre-clinical - Testing a specific compound in the animal
What are the types of validity that should be considered when utilizing an animal model?	Validity • Construct validity - Similar biology  • Face validity - Similar phenomenology (phenotype/behavior)  • Predictive validity - Animals respond to established human drugs 
What are some possible confounding factors when assessing	Confounding factors with cognitive behavior • Motor deficits • Vision deficits • Motivation • Level of activity • Level of anxiety

cognitive behavior?	
When developing a new animal model, what are the advantages and disadvantages of using a series of conventional cognitive tests?	Screening - Multiple conventional tests  • Carry over effect • Order of increased putative impact • Repeat the same test if possible
What are the main reasons to use home cage testing?	Reasons for home cage testing  IntelliCage Lipp (TSE)  PhenoTyper Spruijt (Noldus IT)  Goulding et. al. PNAS 2008  Jhuang et. al. NatCom 2010  PhenoMaster , TSE  SmartCube™ Psychogenics  Gallistel  PhenoRack Viewpoint  HomeCageScan CleverSys • Reduced testing costs • Lack of reproducibility is a major challenge in behavioral neuroscience
What is the PhenoTyper?	Development of automated home-cage methods • Automatic monitoring of behavior - Movement, feeding, drinking

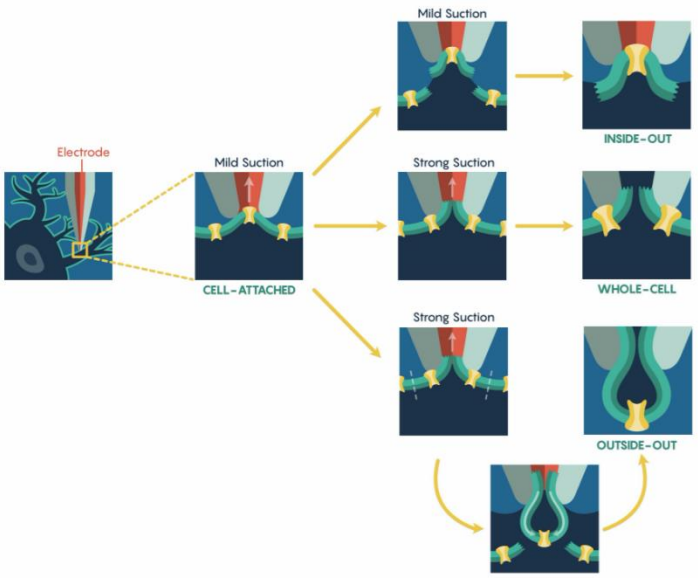
	
Why the tracking of 115 features of mouse behavior would be useful?	Phenotyper - Track the animals with infrared light • Assess of habituation effect, circadian rhythm • Assessment of 115 features   • Facilitates systematic assessment of phenotype - Often a significant difference in a few of the phenotypes will be related to posterior results in cognitive tests
Which strategies could one use to verify rodent behavior in the home cage?	Vibration sensitive setups 
Why doing cognitive tests in the rodents'	Cognitive test with home cage behavior

home cage would be useful?	 • Fear conditioning • Discrimination learning ○ MK801 disrupts synaptic plasticity

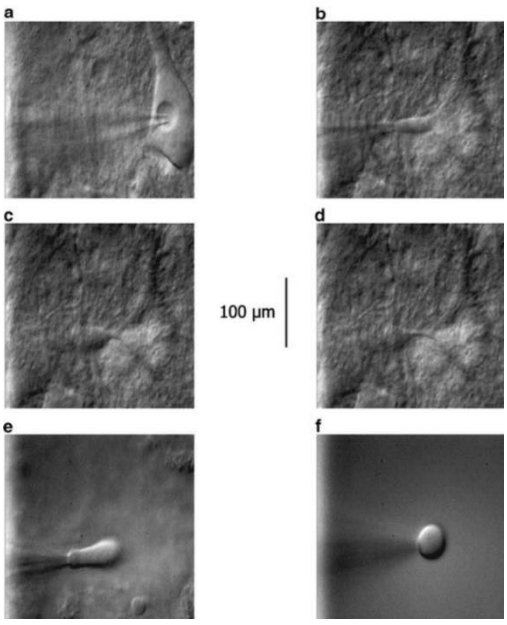
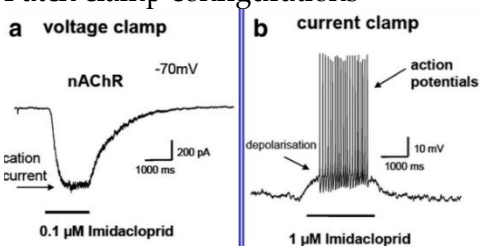
	Not in exam - A manifesto for reproducible science
	• Prevalence of irreproducibility
	  ○ Scientists are motivated to produce as many papers as possible
	
	• Rigorous experimental design
	  ○ Similar body weights across groups
	  ○ Randomize order
	  ○ Blinding of experiments
	  ○ Preplanning the statistical analysis and outcomes measures
	  • Exploratory studies vs confirmation studies -> transparency in reporting (include non-sucessful experiments)

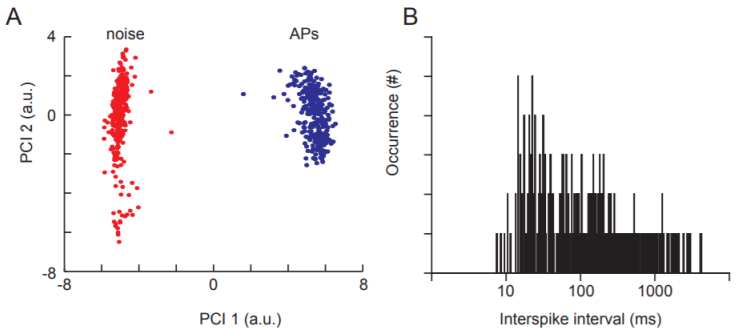
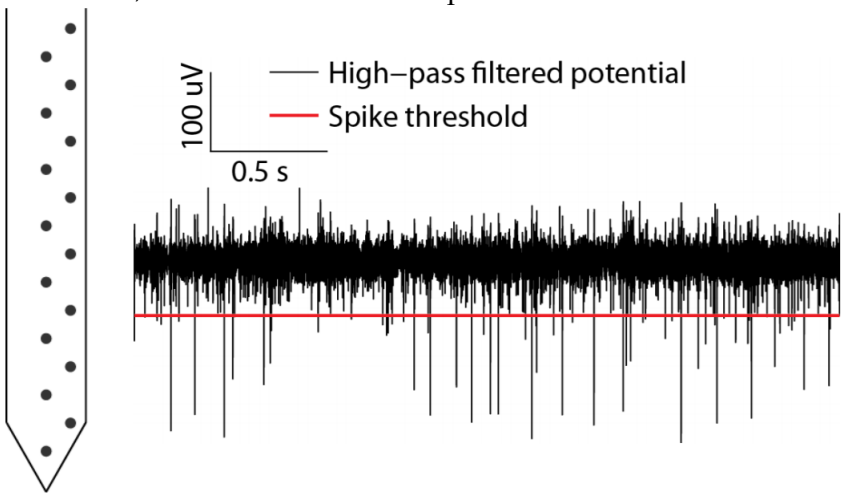
In vivo electrophysiology - possibilities, pros and cons

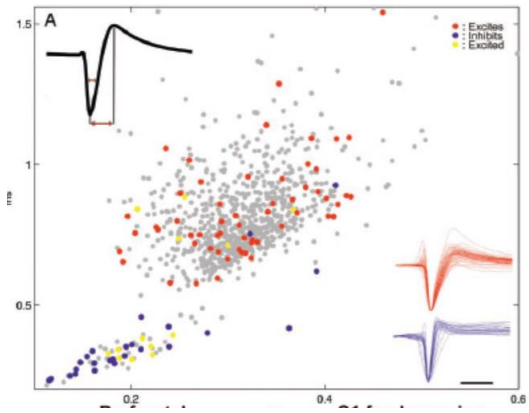
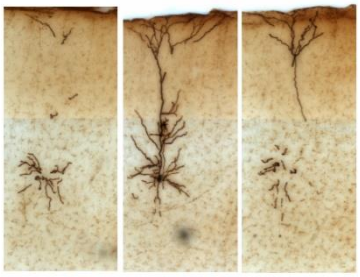
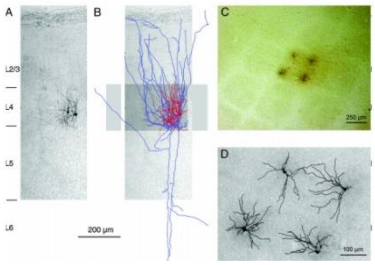
What are the advantages and disadvantages of anaesthetized vs awake electrophysiology?	Electrophysiology
	• Anaesthetized
	  ○ Advantages: stable recordings, easy analysis, long recording
	  ○ Disadvantages: not realistic, anasthesia (different anaesthetics have different influences in the brain), simple information
	• Awake
	  ○ Advantages: realistic, complex information, high impact
	  ○ Disadvantages: unstable recording (even when head-fixed), complex analysis (motor component is also included), short recording

What the three main patch clamp configurations?	Patch clamp configurations
	 
	
	• Electrode is quite thin - 1 micron in diameter
	• Mild suction - 1 giga Ohm
	  ○ Whole cell
	  ○ Inside out - Inside of the patch is the outside of the cell (created with mild suction)
	  ○ Outside out - Outside of the patch is the outside of the cell (created with strong suction)

What is a nucleated patch?	Nucleated patch

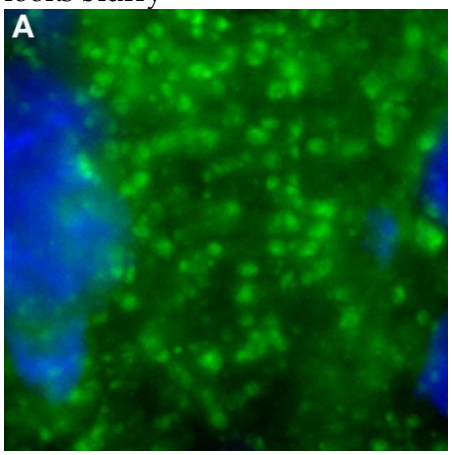
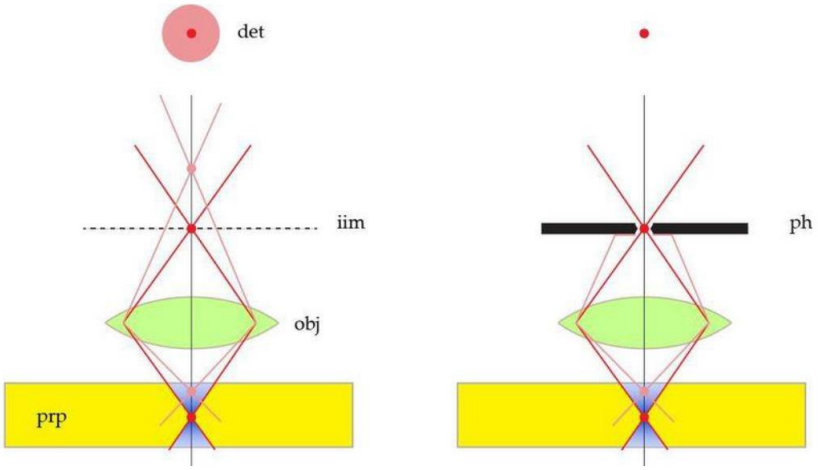
	 • Suck on the electrode - Cell is sucked to the electrode, nuclei is not • Study the cytosol or study the nucleus separately
What is the difference between voltage and current clamp? Which one can be used in vivo?	Patch clamp configurations  • Voltage clamp - Voltage is constant, you study current • Current clamp - Current is constant, you study voltage ○ **In vivo, the only possible strategy is current clamp**
What are the two methods that can be used for in vivo electrophysiology?	*Cortical span of the mouse - 900 um* • *Penetration depth of two-photon - 300 um* *Cortical span of the rat - 2 mm* *Interneurons - Neurons in the same layer* *Inhibitory - Related to function* There are two methods that can be used for in vivo electrophysiology: juxtasomal recordings and ensemble recording
What is the main advantage of juxtasomal recording?	Juxtasomal recording  • Loose suction - A few mega Ohm

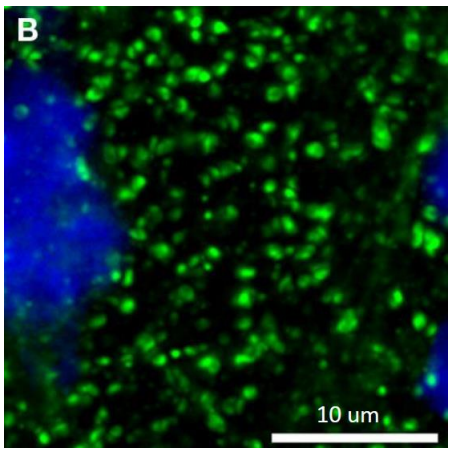
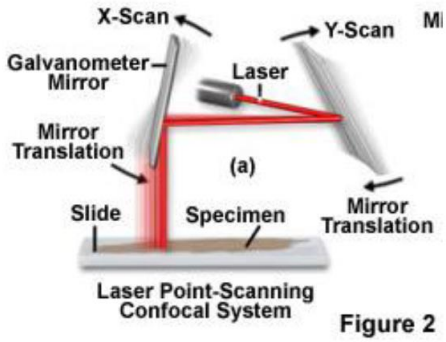
	• Threshold is set on the noise level of the recording  ◦ Define 0% false positive and 0% false negative ◦ Refractory period should not have any spikes - Means that your signal is coming from different cells • Main advantage - You can label the cell to perform validation afterwards
What is the main advantage of ensemble recordings?	Ensemble recordings - 64-channel silicon probe • Filter, threshold and extract spikes  • Multi-unit clustering - PCA (subject to biases) ◦ Phy vs KlustaKwik ◦ Offline routines ◦ Reliability of clustering ◦ Overclustering • You then project the clustered analysis back onto the original data ◦ But you cannot determine where those units are in the brain • Analysis of spike waveform ◦ You can never be sure what cell type it is -> Putative

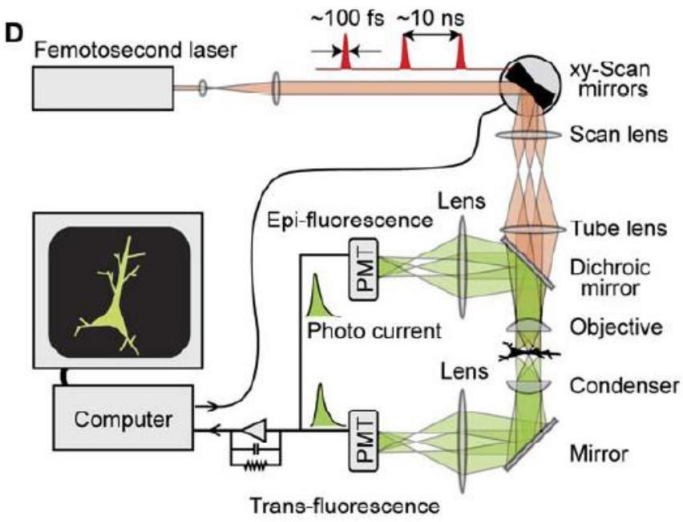
	
What is the difference between in vivo labeling of neurons and in vitro labeling? Which one is better?	Filling & reconstructing the neuron • Dye filling - Made with sequential short pulses (making holes in the neuron which are recovered briefly after) • Labeling the cell to perform validation - Sometimes, different neurons have the same pattern of activity (thin-tuffed and thick-tuffed) ○ In vivo labeling - Reconstruction of different slices can be done in software  ○ In vitro labeling - Histology is restricted to the slice (mainting 85% of dendrites and 15% of axons) 
What are the methods of analysis for ensemble recording?	Methods of analysis • Multi-unit activity - Population-level data clustered together • Ensemble recording - Single unit resolution can be infered by having no spikes in the refractory period • Neuropixels probes - 960 sites to measure activity, 384 that can be used at a time ○ Allows to see patterns in more sparsely distributed population -> e.g. prefrontal cortex

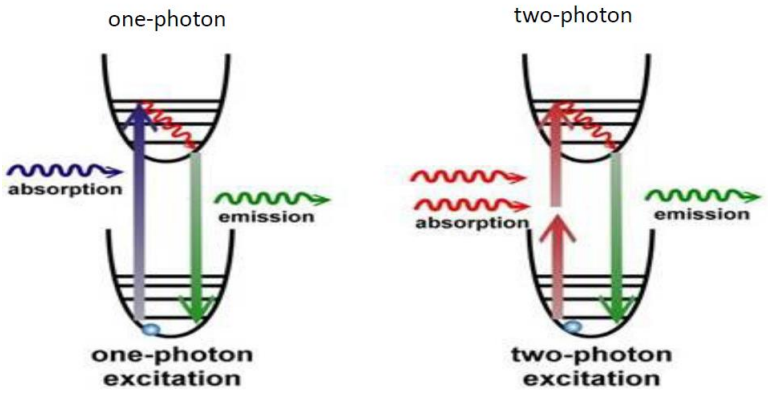
Not in exam	Clustered analysis
	• Klusta - Threshold and event-based analysis of spatiotemporal features
	  ○ Slower, but yields more well-isolated units
	• KiloSort - Threshold and template matching
	  ○ Much more computationally efficient, output data is noisier

Introduction to Fluorescence Microscopy - 1-photon vs 2-photon imaging (Rogier Min)

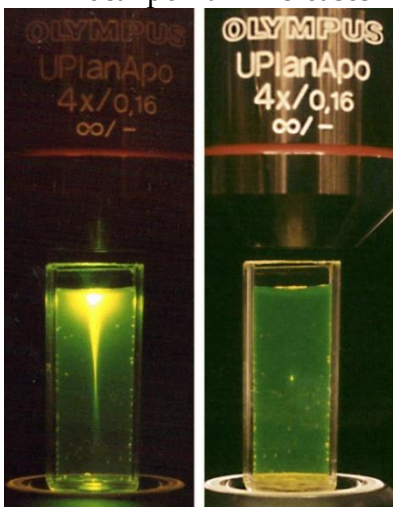
What are the main problems of widefield microscopy?	Widefield microscopy main problem - Limited resolution, staining is often required to see sample features Conventional fluorescence microscopy - High intensity light source needed to activate fluophore; The entire field of view is illuminated Epifluorescence - Background illumination is quite high, image looks blurry 
What is the principle of confocal microscopy?	Confocal microscopy - Aimed to overcome problems with widefield microscopy  • Same principle as pinhole camera - Small aperture -> better focus • Only light that comes from a particular plane of the sample is able to get to the detector - Two points are confocal (same point in the lens)

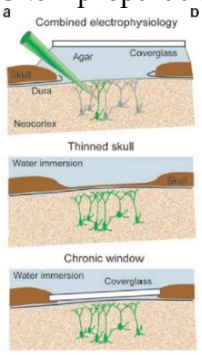
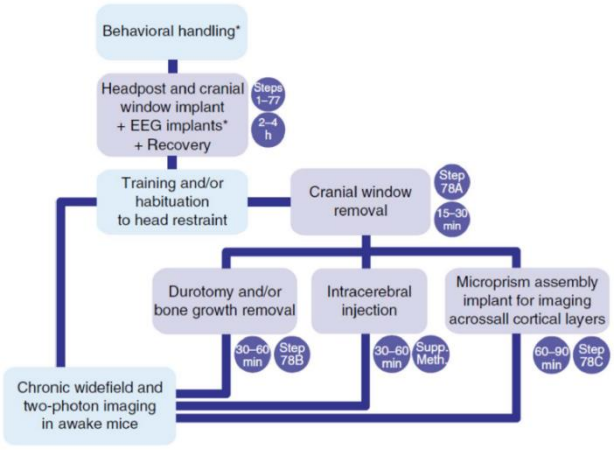
	•  • In order to change the plane, you change the excitation light or pinhole position
What are the possible ways to move the laser in confocal microscopy?	Confocal laser scanning microscopy - XY movement in the sample • Stage-scanning - Stationary laser beam and moving stage platform - Easiest design to achieve • Beam-scanning - Moving laser beam and stationary platform ○ Usually produced by a galvanometer (rotation movement of mirror changes direction of light)  Figure 2 • Acousto-optic deflector: can produce fast (microsecond) jumps of the laser beam -> more accurate than galvanometer • Piezo driven objective scanner or Electrically Tunable Lens - Z movement
What is the principle behind two-photon microscopy? How does it compare to confocal microscopy?	Two-photon microscope

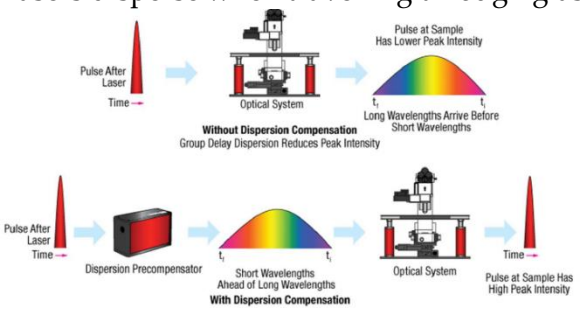
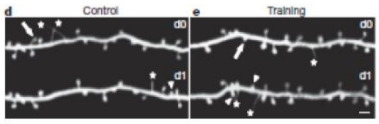
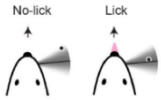
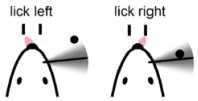
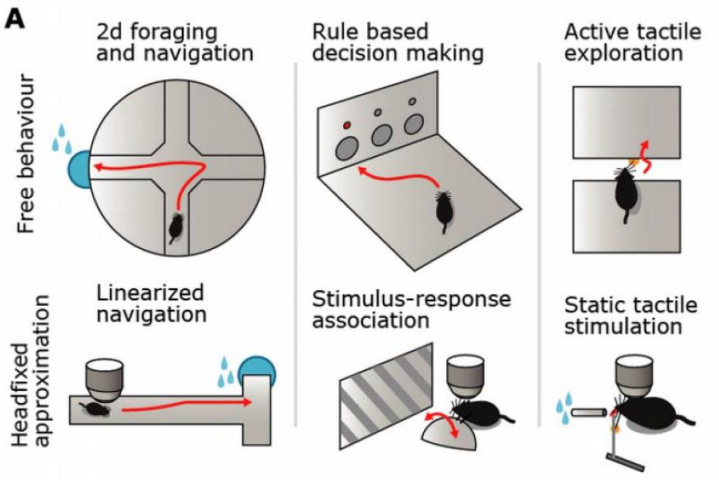


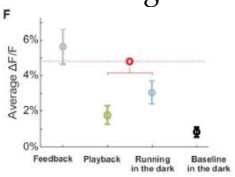
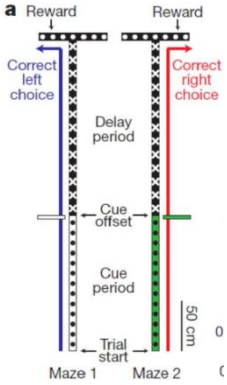
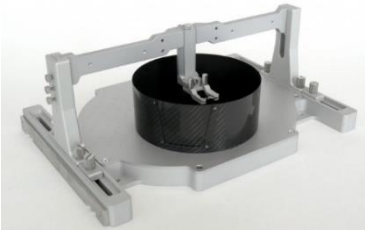
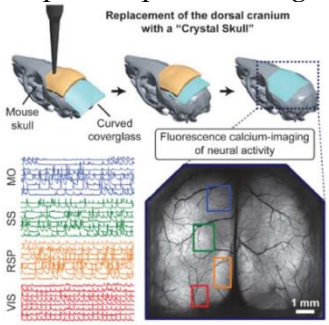
- Femtosecond pulse laser - pulse of 100 fs and 10 ns apart
    - No need for a pinhole - You can look at all the light that comes back
    - The two-photon effect is done with an oscillator - Group continuous emissions into very rapid quanta
- Main difference to a one-photon microscope - Use two photon with half the wavelength, but super quickly and really focused on one point in the sample, are used to excite the fluorophore
- 

    - Changes of two-photon absorption is maximized in your focal point -> increases resolution compared to widefield



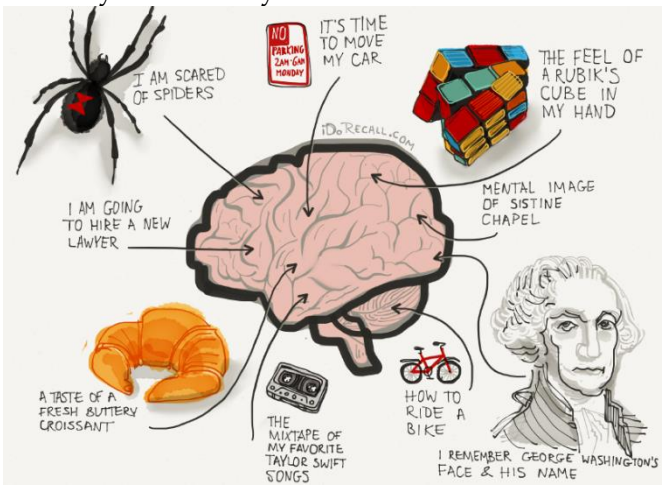
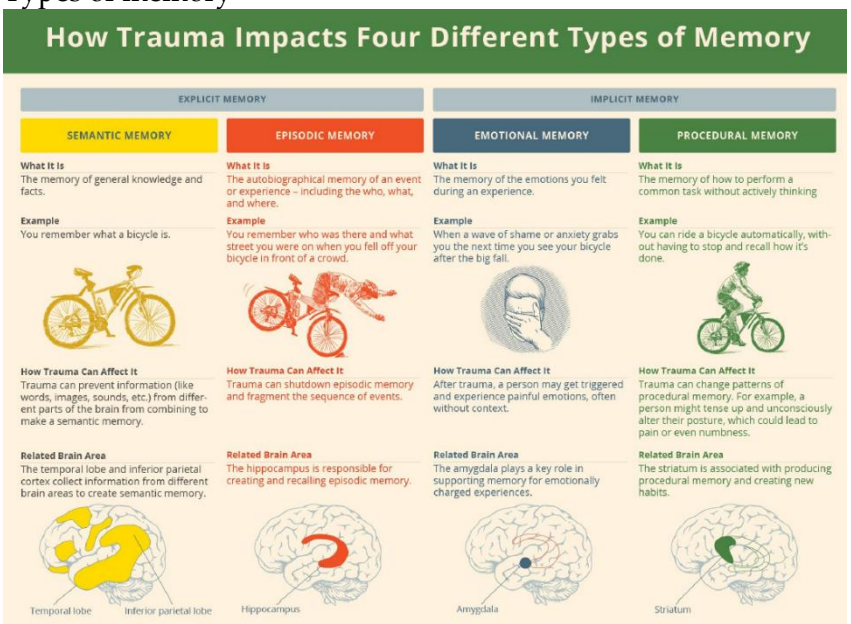
What are the advantages of 2-photon microscopy?	Advantages of 2-photon microscopy Less scattering, it allows to visualize much deeper in the tissue Sample is excited only at focal point - Less photobleaching/phototoxicity
What are the disadvantages of 2-photon microscopy?	Disadvantages of 2-photon microscopy • Expensive • Worse xy resolution compared to 1 photon • Not all dyes/fluorophores work well for 2-photon excitation
What are the methods to prepare the cranial window?	Skull preparations  • Cranial window - Metal post is fixed with dental cement; dental drill is used to cut the bone, cranial window (two glass circles glued together, prevents bone growth)
What is the workflow for in vivo two-photon microscopy?	Workflow 
What are the main learning points from the history of 2-photon microscopy?	History 2-photon miniscope • Helmchen (2001) - 25 g 2P miniscope; only rats could carry it around • Problems with 2-photon miniscope compared to 1-photon - Heating (higher energy); more movement artefacts

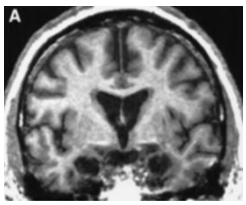
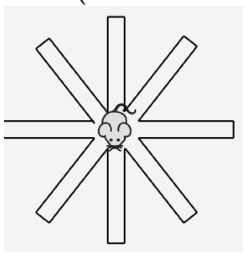
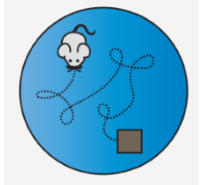
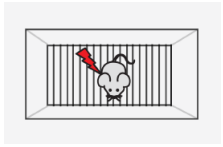
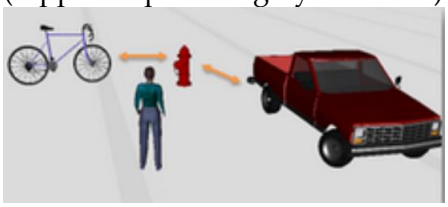
How could a fast two photon pulse be transmitted by a fiber, if the change in medium causes a spread in the wave (decreasing intensity and increasing the time)?	Lasers disperse when travelling through glass  • Long wavelengths arrive before short wavelengths • Compensator: Makes long wavelengths slower, arrive at the same time as short wavelengths
What types of experiments can be done with head fixed animals?	In vivo 2-photon with head fixed animals • Anaesthesia  ◦ Spines formed after learning • Awake mice ◦ Need to be motivated with water/food restriction •  ◦ Licks in go/no-go experiment (pole touching a certain position of the whiskers) • Lick/no lick is too simple - Lack of behavior can be interpreted as many different ways, left/right lick is better to distinguish an error vs an omission • 
How could add movement to a head-fixed two-photon setup?	Adding movement 

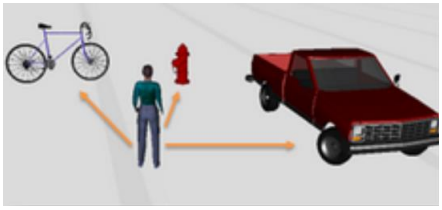
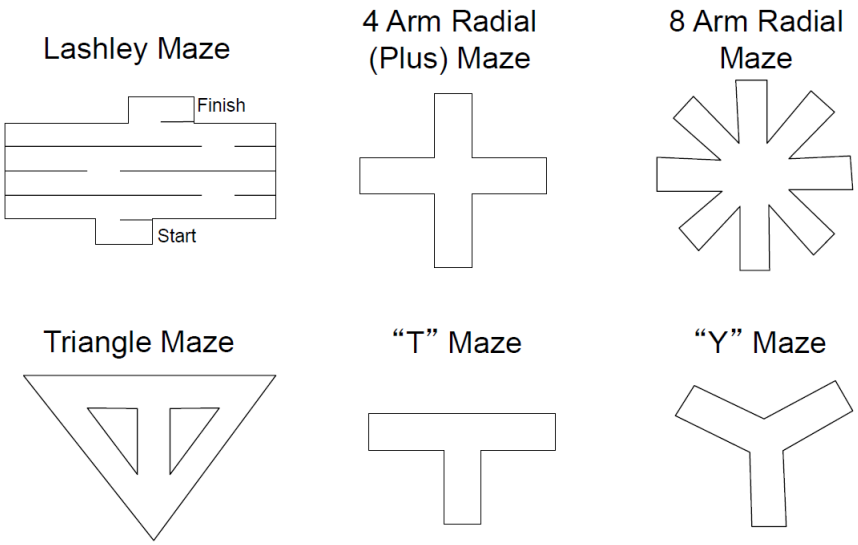
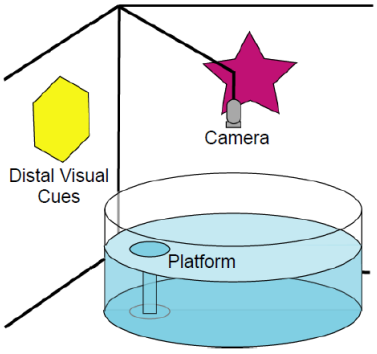
	• Adding movement changes neuronal activation of the primary visual cortex (running in the dark yields more activity than just looking at a moving image)  Virtual reality - Allows for complex decision-making/memory tasks •  • Can also be combined with pupil tracking -> make sure where the animal is looking Mobile home cage - Animal moves with environment 
What is mesoscope two-photon imaging?	Mesoscope two-photon imaging •  • 5 mm of brain imaging • Replacement of the whole skull with a piece of glass
What is the advantage of voluntary head fixation?	Voluntary head fixation  • More naturalistic behavior

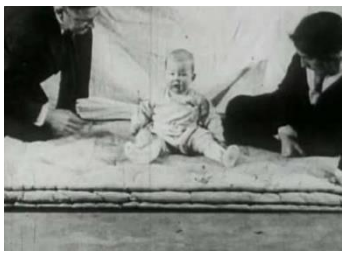
What is the advantages and disadvantages of mobile/immobile two-photon setups?	Summary Advantages: • Head-fixed - Better resolution (subcellular structures) • Movements artefacts are limited • Many cells simultaneously • Sensory stimuli can be well control Disadvantages: • Invasive surgery • Habituation is necessary • Behavior repertoire is quite limited • Artificial vestibular inputs

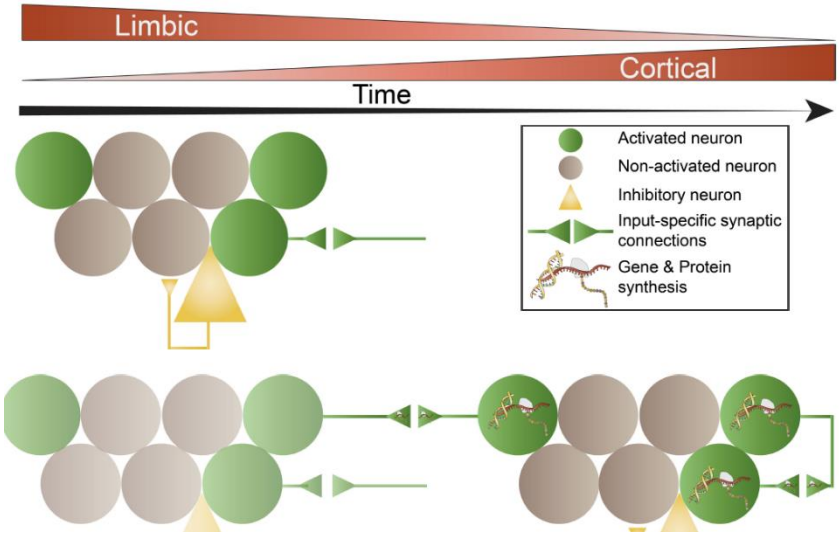
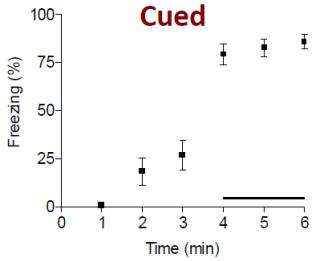
Learning and Memory (Priyanka Rao-Ruiz)

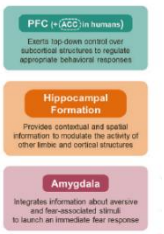
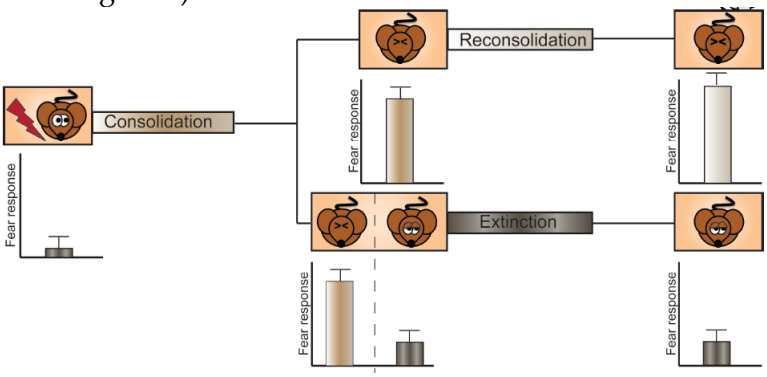
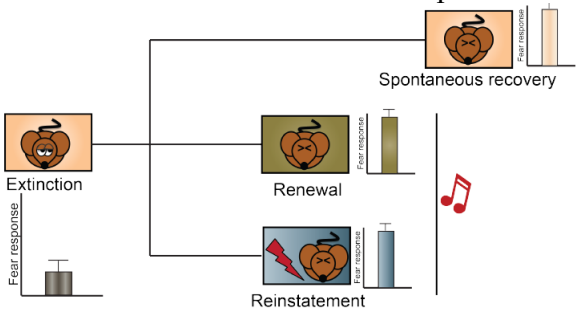
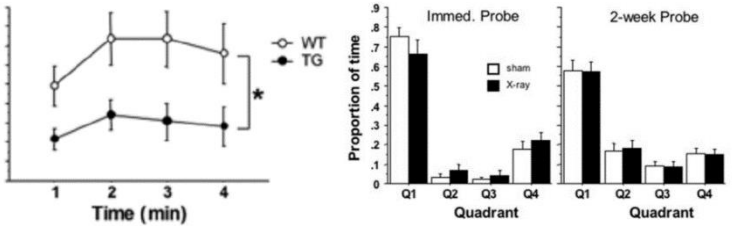
What is memory?	Memory - Process by which information is encoded and stored  • Can be aversive, neutral or rewarding • Required to adapt in changing environment
What are the four types of memory?	Types of memory  • Semantic - General knowledge and facts • Episodic - Autobiografical • Emotional - Emotions • Procedural - Motor skills
Who was patient HM?	A lot of knowledge of memory is drawn from trauma patients • Patient HM - Bilateral hippocampal lesion, leads to anterograde amnesia

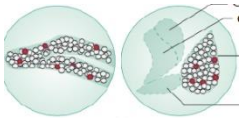
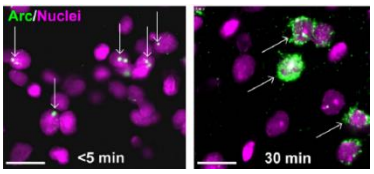
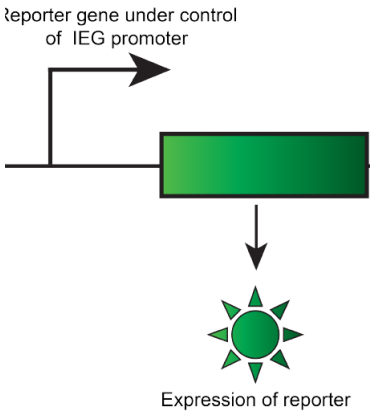
	
Which aspects should you consider when choosing a memory task?	Choosing a memory task • Depends on the goal of the study? Kind of memory/brain region/disease? • Does your chosen behavior have validity? • Which animal model? ○ More genetic tools are only available for mice, not rats • Is the animal model compatible with the task/intervention techniques?
Mention 3 behavior paradigms that assess memory (and which type of memory)?	Behavior paradigms: • (8-arm radial) maze - Working memory, spatial memory  • Morris water maze - Long-term spatial memory  • Fear conditioning - Long-term aversive memory 
What are two different types of spatial memory?	Spatial encoding system • Allocentric - Reference from objects to other objects (hippocampus is highly involved)  ○ If you want the hippocampus to be involved, you need to place cues around the task • Egocentric - Reference from objects to self (hippocampus is not highly involved)

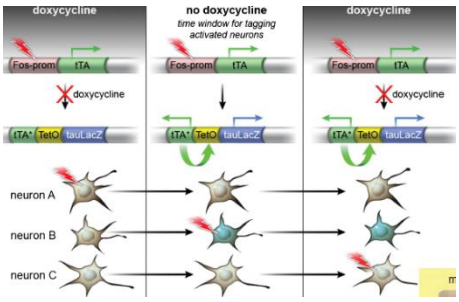
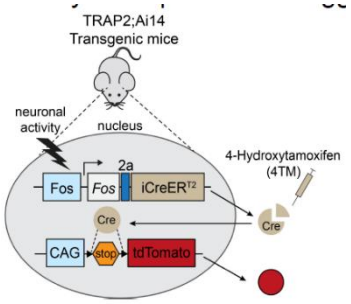
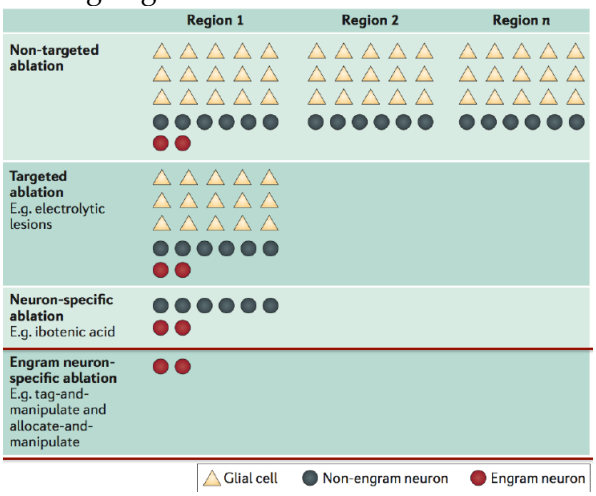
	
What is the main disadvantage of using mazes as a memory test?	Rodent mazes  • Disadvantage: Animals need to be food restricted
What are the main advantages and disadvantages of the Morris water maze test?	Morris water maze - Animals are equally motivated to leave the rats, no food restriction, efficient learning (compared to mazes)  • Measurements: Time to reach platform, time spent in quadrant • Advantages over mazes: No food restriction, all animals complete the task, well defined allocentric learning, species specific response • Disadvantage: Cannot test working memory, may be unduly stressful
What is the fear conditioning paradigm? How can you either test cue	Fear conditioning - Very short training, persistent memory

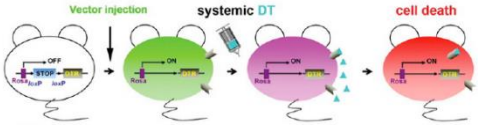
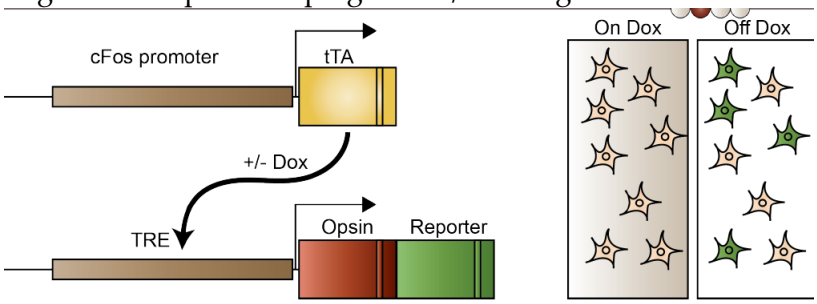
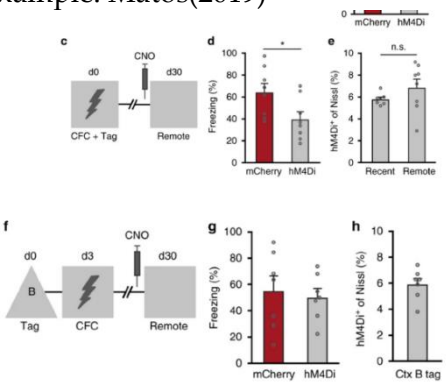
memory or context memory?	
	• Beginnings - Little Albert, generalized fear response to fluffly white things
	• Animal model:
	  ○ Tone - Conditioned stimulus; cue memory is amygdala driven
	  ○ Shock - Unconditioned stimulus; context memory is hippocampus driven

How memory is consolidated at different time points after an event occurred?	Consolidation of memory  • Initially synaptic consolidation ○ Requires gene transcription/protein synthesis • Later systems consolidation
How is the fear memory measured? Which brain regions are involved?	Measurement of fear = freezing  • Brain areas encoding fear memory

	• 
Define the following processes: 1. **Retrival** 2. **Reconsolidation** 3. **Extinction** 4. **Renewal** 5. **Reinstatement**	Memory processes: Retrival/consolidation (long term memory storage), reconsolidation (cued recall of the original memory), extinction (reduced fear response when CS is not followed by US for a long time)  • Reconsolidation can be targeted with drugs: propanolol removes the emotional component of a memory • Extinction - Used to be used for therapy, however extinction does not erase the original memory, so it can always be retrived (renewal = in a different context and reinstatement = when the aversive stimuli is presented again) 
What does the ablation of neurogenesis in DG leads to in 1) fear conditioning and 2) water maze test?	Neural substrates of contextual and spatial memory are not the same  • Ablation of neurogenesis in DG impairs contextual memory (in fear conditioning), show no effect for cued memoy (water maze test)

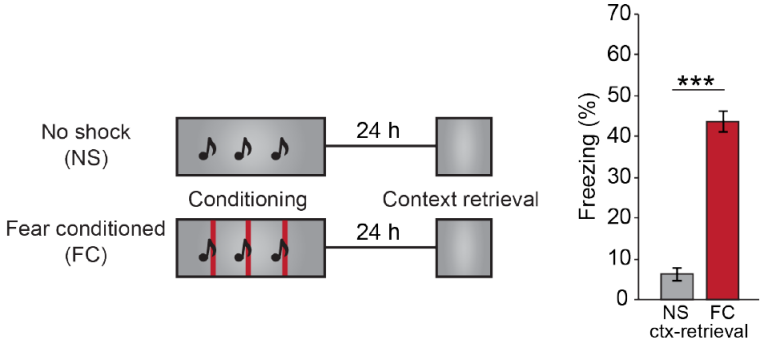
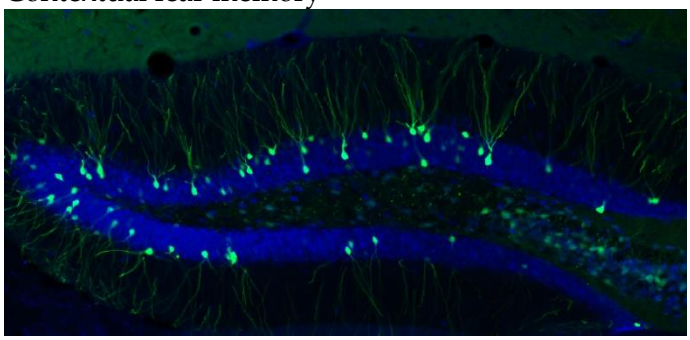
What are memory engrams?	Memory traces/engrams • Small population of neurons that form the physical substrate of the memory 
What are the four aspects that define a memory engram?	Defining an engram • Persistence: an engram is a persistent change in the brain that results from a specific experience • Dormancy: not active when the memory is not active • Ecphory: Expressed behaviorally through interactions with retrieval cues • Content: Engram predicts what was encoded and what will be retrieved
How can you observe an engram with immediately early genes? What are some examples of IEG?	Observing engram: • Immediately early genes - Immediately activated after the neuron is active  ◦ Transcription factors - c-fos, zif268 ◦ Structural proteins - Arc, homer1a
What would the constructu Arc::dVenus allow you to visualize?	Introduction of virus with IEG and a fluorescent marker (Arc::dVenus) • Arc is only expressed in neurons that express CaMKII (glutamatergic) • Neurons that were active will express GFP variant • Tagging is not specific • 
Describe the tet-tag system.	Tet-Tag systems

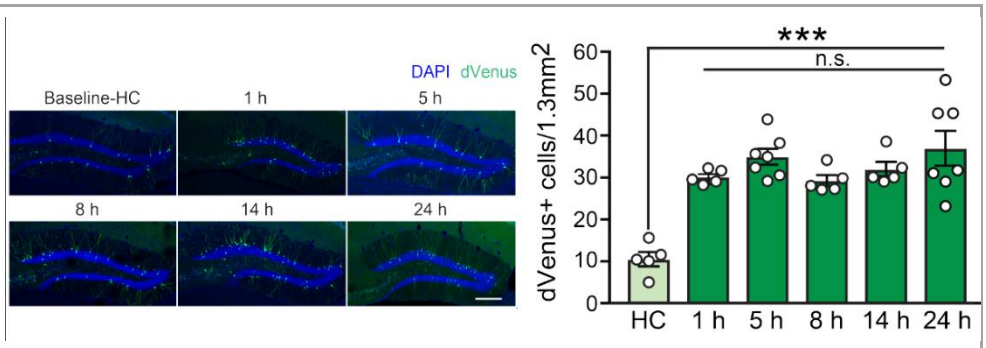
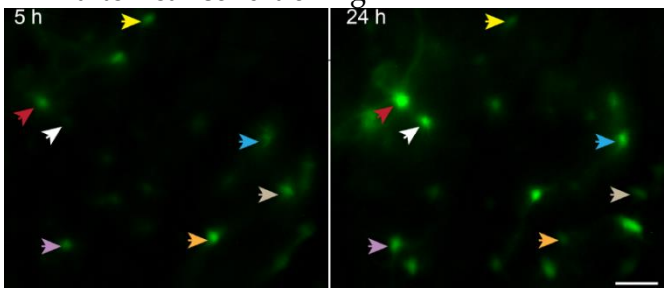
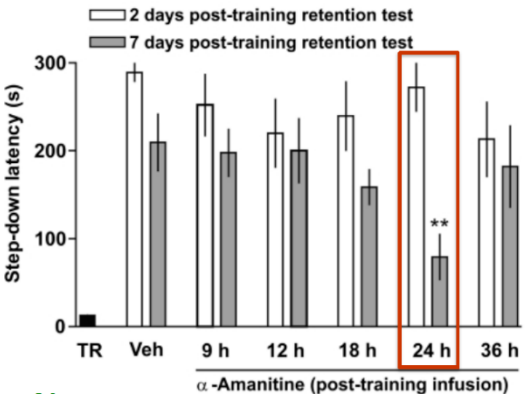
	 • Doxycycline blocks expression of LacZ(or GFP) • When the animal stops eating food with doxycycline opens a window of tagging • When the animal starts eating food with doxycycline again, the window of tagging closes
Describe the TRAP system.	TRAP2 system (targetted recombination in active populations) • iCreERt2 - Tamoxifen dependent cre -> recombination only occurs in cells when tamoxifen is present ○ Cre goes out of the nucleus when fos is active • 4-hydroxytamoxifen injection -> promotes cre translocation into the nucleus •  • The tagging is **permanent**
Under which levels of precision is it possible to erase an engram?	Erasing engrams  • Engram neuron-specific ablation ○ More excitable neuron -> express CREB

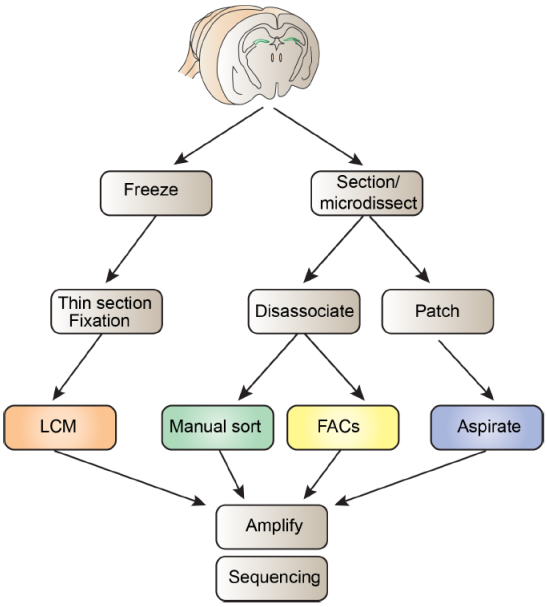
	○ Colocalization of Arc and CREB -> Neurons have a higher probability of becoming part of the memory engram
Describe how diphteria toxin can be used to only kill neurons that are part of an engram.	CREB-Diphteria toxin receptors  ○ Cells that are part of the engram are selectively killed
How is it possible to only express opsins/DREADDs in neurons that are part of an engram?	Tag and manipulate: Optogenetics/Chemogenetics  • Tta -> leads to the expression of the opsin
Which control could be used to make sure that the engram is related to the fear memory (and if you optogenetically inhibited a random population of neurons you would observe the same effect)?	Example: Matos(2019)  • Control experiment: Put mice in a novel context and tag neurons; only after a few days you do the fear conditioning
What are the main limitations of fear conditioning and how could they be overcomed?	Limitations: - Overtagging -> Homecage control is necessary • Only freezing behavior is observed as a downstream effect of the memory -> more complex tasks can be used (sequence learning) • Selective targetting of a broad network (optogenetics) -> DREADD can be expressed in multiple brain regions
	Conclusion

	✔**Persistence:** an engram is a persistent change in the brain that results from a specific experience or event ✔**Dormancy:** An engram may exist in a dormant state between the two active processes of encoding and retrieval 3. **Ecphory:** An engram may be expressed behaviorally through interactions with retrieval cues 4. **Content:** The content of an engram reflects what transpired at encoding and predicts what can be recovered during subsequent retrieval • IEG/IEG promoters useful tools to tag, characterize and manipulate memory traces • Memories can be manipulated at time points remote from encoding when the likelihood that these engrams are being actively processed is low 1. **Persistence:** an engram is a persistent change in the brain that results from a specific experience or event 2. **Dormancy:** An engram may exist in a dormant state between the two active processes of encoding and retrieval ✔**Ecphory:** An engram may be expressed behaviorally through interactions with retrieval cues 4. **Content:** The content of an engram reflects what transpired at encoding and predicts what can be recovered during subsequent retrieval Stimulation/inhibition of engram neurons leads to involuntary bi-directional modulation of memory expression, addressing the ecphory criterion 1. **Persistence:** an engram is a persistent change in the brain that results from a specific experience or event 2. **Dormancy:** An engram may exist in a dormant state between the two active processes of encoding and retrieval 3. **Ecphory:** An engram may be expressed behaviorally through interactions with retrieval cues ✔**Content:** The content of an engram reflects what transpired at encoding and predicts what can be recovered during subsequent retrieval • Artificial reactivation of captured engram neurons and physical presentation of an external retrieval cue seem functionally equivalent, but there are differences • The neurally reinstated behavioural response is smaller in magnitude than the naturally reinstated response

Molecular approaches in memory research (Priyanka Rao-Ruiz)

How does the valence change the strength of a memory?	The valence strengthens a memory - Strongly rewarding or strongly aversive
What is fear conditioning?	Contextual fear memory Control - Tone is present without a shock Experimental group - Three tone/foot shock pairings  Results in robust and persistent fear memory
What is memory consolidation ? Which brain regions does it involve?	Memory consolidation - Transition between short term (hippocampus-dependent) and long term memory (cortex dependent) • Depends on gene and protein transcription
Which percentage of neurons in the dentate gyrus are involved in a fear memory?	Contextual fear memory  • 2-6% of DG granule cells are activated and involved in memory
What are the main disadvantages of the Arc:dVenus system?	Arc::dVenus expression

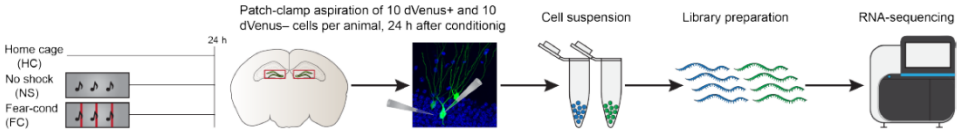
	 • Sustained expression specific for the dentate gyrus • Every point is an individual (journals are starting to ask for data at an individual level) • Disadvantages: not inducible system/neurons of different animals at different time point
How could you prove that the same neurons are active 5h and 24h after fear conditioning?	Solution: Miniscope • The same population of cells (79.81%) remain active up to 24 hours after fear conditioning 
What is alpha-amanitine? How can it interfere with fear conditioning?	Ama/Veh - Transcription blocker  • Delayed transcription is required for late phase of consolidation (not 24 hours later, but 7 days later)
What are some methods to harvest tagged neurons?	Methods to harvest tagged neurons



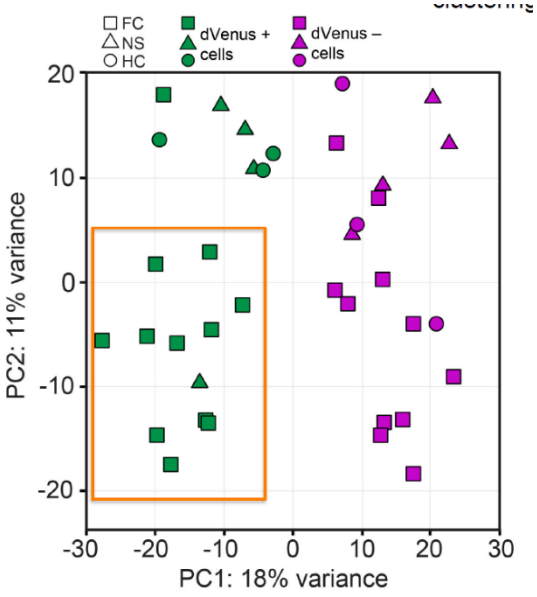
- LCM - Laser capture microdissection
- FAC - Fluorescent active cell sorting
  - Takes 4-5 hours - RNA degrades, cells may die during the processing

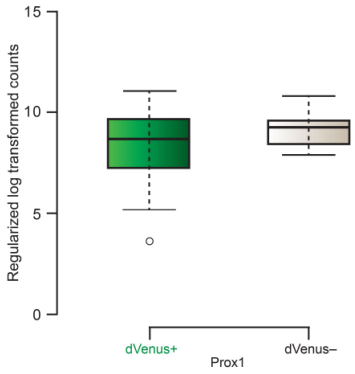
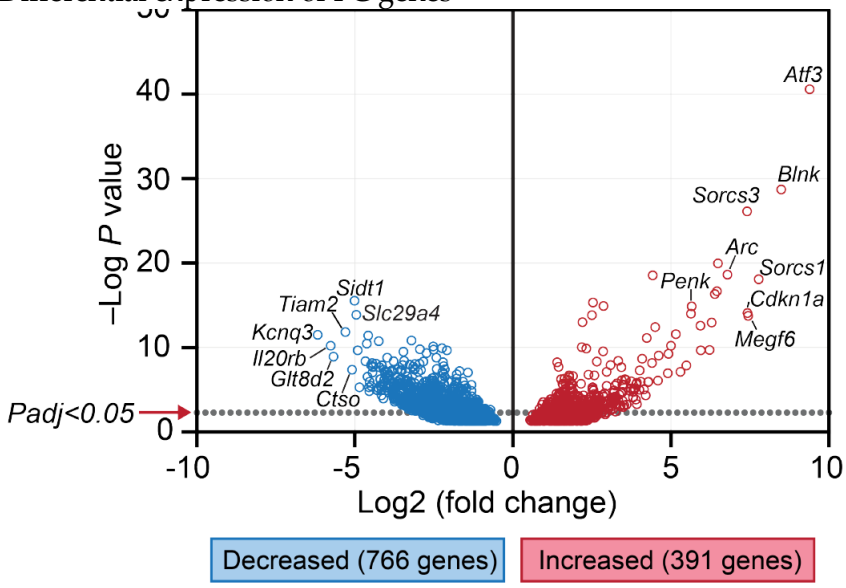
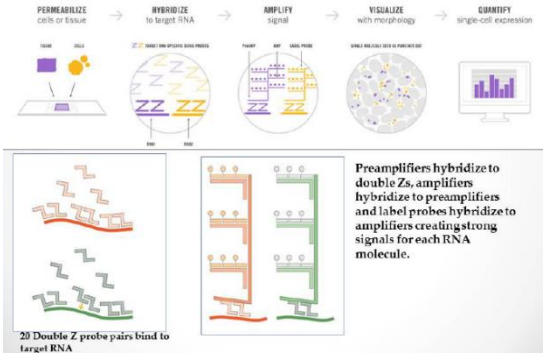
What are the groups used in aspiration of tagged neurons? Why would you use Prox1 as a control?

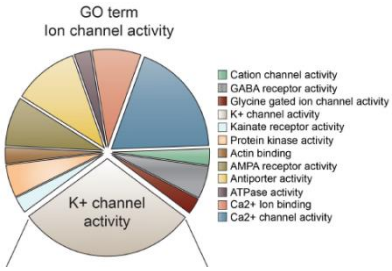
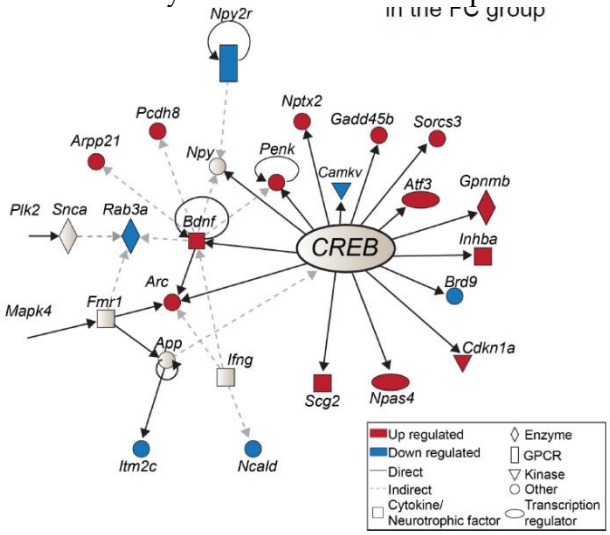
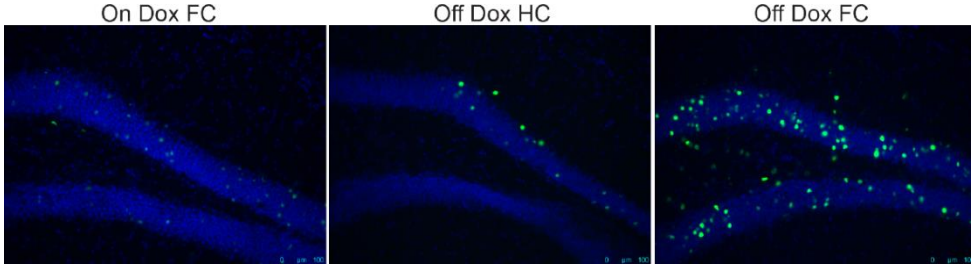
Method of choice of paper - Aspiration

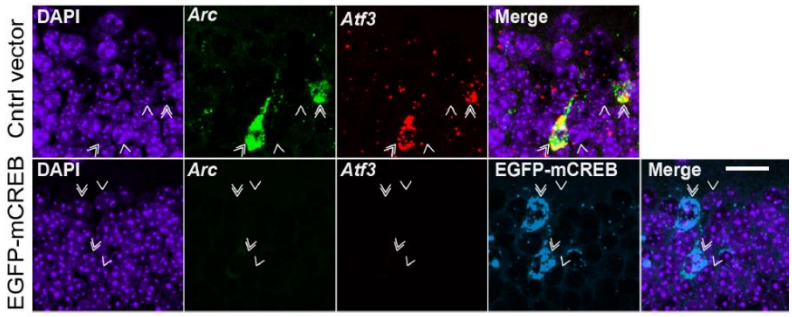
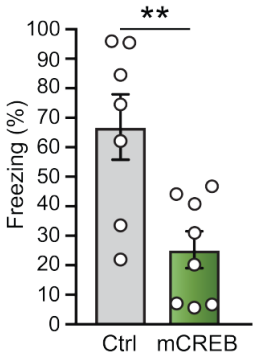


- 10 dVenus cells, 10 neighbouring cells as a control
- RNA sequencing
- Results: Clustering by state of activation; clustering by fear memory ensemble neurons

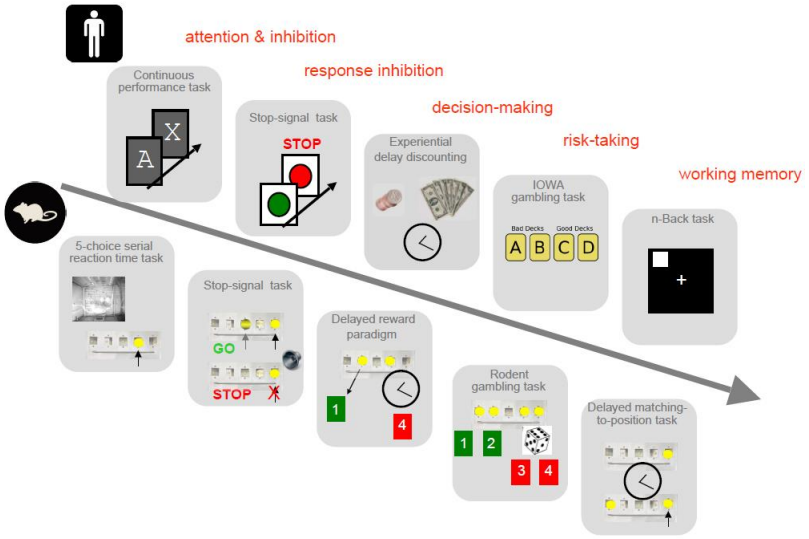


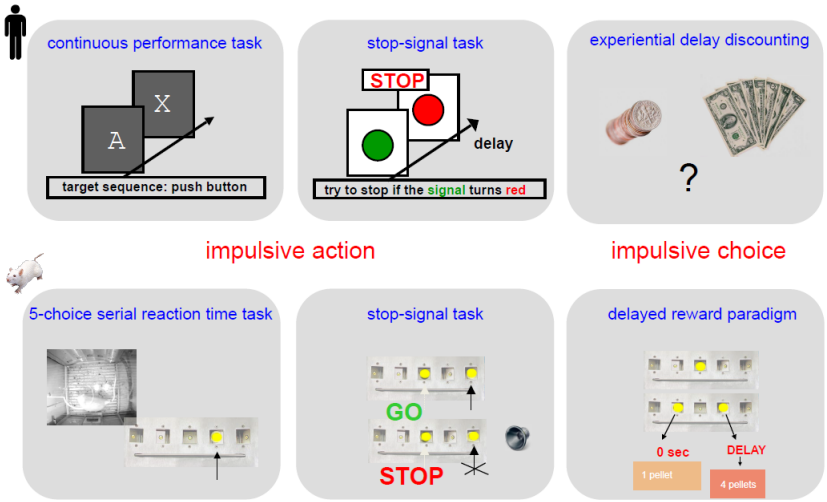
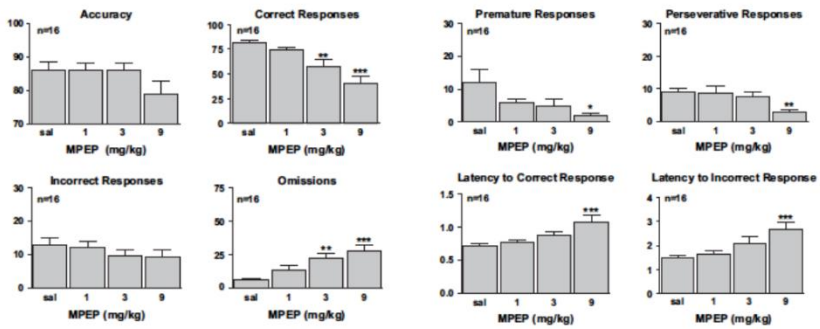
	• Controls: Prox1 - Only expressed the dentate gyrus cells; markers for non-neuronal cells 
What is the advantage of using single cell sequencing?	Differential expression of FC genes  • These results would never be picked up be whole tissue analysis
What is the RNAscope? How is the signal amplified?	Validation of identified gene expression  • RNAscope - Thin slices (10 um), hybridize RNA with probes (many individual fluorescent molecules bound together) -> signal amplification, visualize with morphology
Which genes are more	Over-representation of ion channel pathways

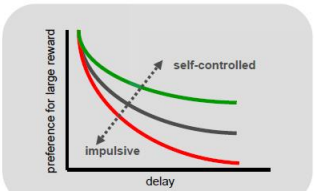
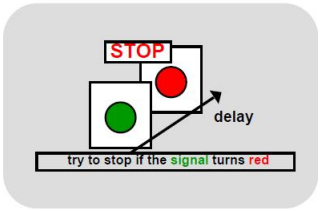
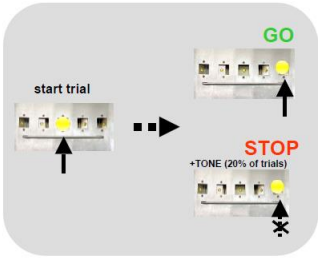
active in the engram neurons?	
What is the most common gene expressed in engram neurons? Why is this a problem?	Network analysis - Common transcription factor = CREB  • CREB binds to cAMP response element (CRE) of genes -> Acts as a transcriptional activator when phosphorylated • Problem: Most studies use CREB-KO -> is the effect an engram effect or a network effect?
Which system would you use to study the effect of CREB in engram formation?	Disrupt CREB **after** the formation of an engram • Arc system could not be used: it is not inducible (cannot be turned on and off) • Use of fos system • CREB levels remain the same - Function is driven by phosphorylation, not expression ○ Use of dominant negative constructs (mCREB) - Similar proteins that bind to endogenous ligand, prevent normal function
What is mCREB?	Results: • Groups  • In cells that express mCREB, Arc and Atf3 are not expressed

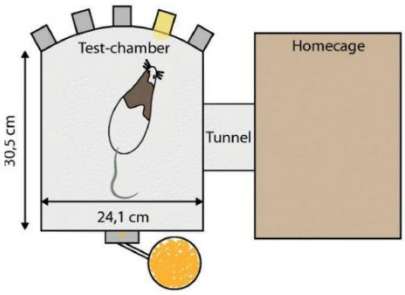
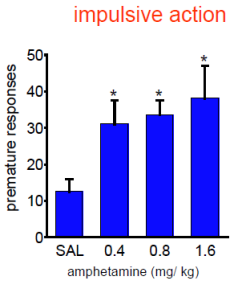
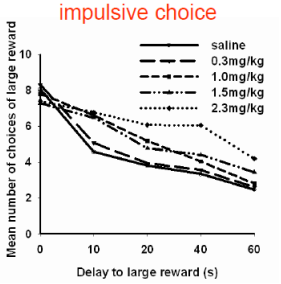
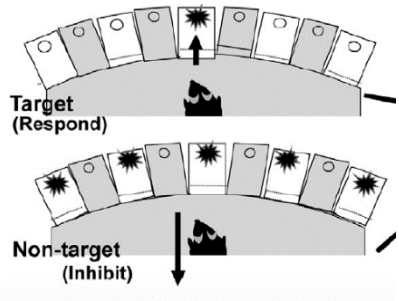
	 • No effects on acquisition, no effects in STM, significant effect on long-term memory  ○ Keep in mind that you should use different animals for every time point -> prevent confounding effects of reconsolidation
What are current limitations of molecular studies of engrams?	Discussion • Gene expression vs protein expression? ○ Protein turnover ○ Post-translation modifications • Memory substrates -> most probably not present in the soma • It is possible to look at proteins, but proteomic techniques are still not very sensitive compared to RNA-seq • Are all memories encoded by the same molecular pathways? ○ Hum…

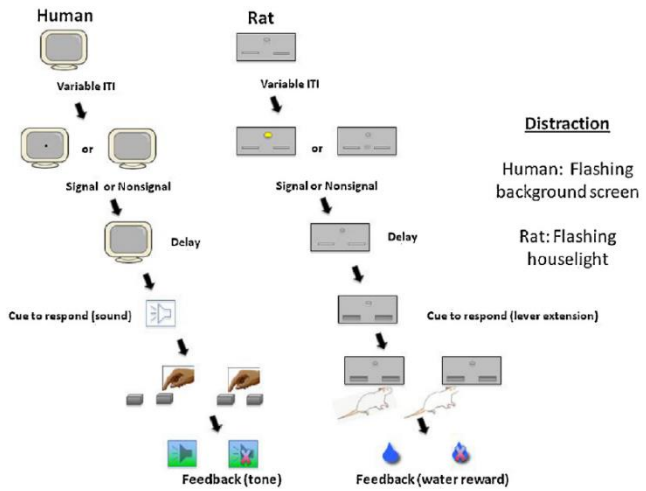
Translational models of impulse control and attention (Tommy Pattij)

What is the current toolbox to study impulsivity and attention?	Neuropsychological toolbox to understand cognitive functions 
What are the methods of intervention when studying cognitive functions?	Methods of intervention 
What is impulsivity? Why studying impulsivity is relevant?	Maladaptive impulsivity is observed in some neurological disorders, such as addiction, ADHD, OCD • Definition: Actions that are poorly conceived, prematurely expressed and innapropriate for the situation, leading to undesirable consequences
Which tests can be used to study impulsive action/impulsive choice?	Measuring impulsivity in humans • Self-reported: BIS (Barratt impulsiveness scale) • Behavioral: Stop-signal, go-no go

	 • Impulsive action: poor inhibitory control (assessed in 5CSRTT) • Impulsive choise: poor decision making (assessed in delayed reward task)
What is the 5CSRTT and what does it measure?	5-choice serial reaction time task (attention and impulsivity) • Adaptation from continuous performance task in humans • Attention is measured by task accuracy, impulsivity is measure with premature responses ○ There needs to be a negative consequence for the premature response
Exam question: what conclusions can be drawn from the following data?	mGluR5 antagonist MPEP in 5CSRTT  • Main effect: dose-dependent directional effect on accuracy
What is the delayed reward task and which cognitive process does it measure?	Delayed reward task • 1 pellet immediately or 4 pellets with a delay (5, 10, 20, 40)

	   Translational approach to study impulsivity • High variability in humans
What is the stop-signal task? What is the race-model?	The rat stop-signal task   • Start with go stimulus and turns into a stop stimulus (when a beep comes up, the rat needs to disingage with nose poke) • Race-model: captures which time of stop activation generates a 50% chance of making a correct or incorrect response
What are the advantages and disadvantages of animal models for impulsivity and attention?	Advantages: • Translational model - cross species compatible • Good construct and predictive validity • Allows for within subject experiments (overtraining is still possible though) Disadvantages: • Ethologically relevant task? • Extensive periods of training • Performance driven by reinforcement (food restriction)
What are the future of home cages and how will they improve	Future of home cages

the quality of studies with animal models?	 • Socially housing animals (rats are social creatures)
What is the role of dopamine in impulsive action and impulsive choice?	Role of dopamine in impulsivity • Amphetamine increases impulsivity in 5CSRTT and decreases impulsive choice in delayed reward task •   ○ Mediated by D2 receptors in the Nucleus accumbens core (Pattij 2007)
What is the 5CCPT?	5-choice continuous performance task  • Non-target trials - All five holes are illuminated, the animal should inhibit a response
What is the sustained attention task?	Sustained attention task

	 • Signal or non-signal trial - The rat needs to indicate which one it was by doing different lever presses
Why is optogenetics useful to study attention and inhibition?	Optogenetics • Optogenetic modulation of impulsive behavior