Neuronal basis of behavior

By Hana Brozka

"Man can do what he wills but he cannot will what he wills."
— Arthur Schopenhauer, Essays and Aphorisms

Outline

- What is behavioral neuroscience?
- Tools to study neuronal basis of behavior
  - Tools to modify neuronal activity/function and their pitfalls
  - Behavioral tools and their pitfalls
- Exploratory behaviors - spontaneous alteration
- Goal directed (motivated) behaviors
- Pavlovian associations
- Reinforcement
- Schedules of reinforcement
- Habit fomation
- Stereotypical behaviors
- Addictions
- Social behaviors - social approach, aggresivity
- vocalizations

What is behavioral neuroscience?

- is the study of the biological basis of behavior in humans and animals
- covers a range of topics, including genetic, molecular and neuroanatomic substrates of behavior, neuropsychology, learning and memory, motivation and emotion, and sensory processes
- studies the interplay between the brain, behavior, and the environment
- Behavioural vs cognitive neuroscience: behavioural pertains to movement, cognitive to thought

Tools to study neuronal basis of behavior

- Behavioral tools
- Tools to interfere with normal brain function
    - Administration of agonist/antagonists (systemic, localized)
    - Lesions (permanent)
    - Inactivation (temporary)
    - Optogenetics
    - Chemogenetics
    - Genetic models (knock outs, inducible knockouts (dox on dox off)
    - Transcranial magnetic stimulation
- Tools to observe undisturbed brain activity
    - Immediate early genes
    - Electrophysiology
    - Calcium imaging
    - MRI, PET, EEG
    - Spatial NGS

Pitfalls of presently used tools in behavioral neuroscience

- Behavioral tests:
  - Rarely test assesses only one behavioral 'entity' (differential state of attention, anxiety, motivation, arousal all can impact a results of the study)
  - Usually a single parameter is selected to measure behavior. If more parameters are selected (most often) **inapproprate statistical methods** are used (MANOVA = right; several ANOVAs = wrong - increases posibility of false positives (type 1 error) and disregards relationships between output variables)

- Interferance with normal brain function:
  - Chronic inactivation of brain regional activity/genetic models: **compensatory mechanisms** may develop (both behavioral and in neuronal circuitry). Genetic models are ok when they are genetic model of geneticaly based disease (because persumably the same compensatory mechanisms are present in patients as well)
  - Acute inactivations/facilitations of brain regional activity (muscimol, optogenetic, chemogenetic): can **altered state can divert atttention of the animal** ('feeling stange') - habituation to the manipulation prior to the experiment is therefore essential

- Observation of neuronal activity:
  - IEG expression: only neurons that undergo neuroplastic changes are stained, very **low temporal resolution**
  - Electrophysiology: relatively **small areas** can be observed at the same time (but very good temporal resolution
  - Calcium imagining: larger areas can be explored, with worse temporal resolution (compared to electrophysiology) deep structures are more difficult to asess (GRIN lens inplantation is needed)
  - MRI, PET - generally **low temporal resolution** in rodents
  - PET, EEG - **low spatial resolution**

Innate vs. learned behaviors: innate

- Innate behaviors do not require learning

- 'instinct'

- Appears in fully funtional form the first time, and are expressed even when the animal is raised in isolation

- Important in survival of the individual and propagation of species (feeding, defence, parental care, ociability in social species)

- Innate behaviors are complex

- Species-specific

- **Hypothalamus** is essential for expression of innate behaviors (four F's": fighting, fleeing, feeding, and mating)

- It was difficult to study, nuclei are very interconected and each nuclei contains different groups of neurons responsible for different functions- finally, more selective methods avaliable in the last decade

- Common principles: **integratory hub, redundancy and neuronal population with antoagonistic function within the same nucleus** (recieve same inputs, project to same areas but use different neurotransmitter to convey opposive signal)

- **Antagonistic control** is a common theme to maintain homeostatis (sympaticus vs parasympaticus – same organs are innervated and different neurotransmitters convey opposite signal, insuline vs glucagon, postural stability: biceps v. triceps). Helps to maintaining state of the animal within narrow homeostatic range

Innate vs. learned behaviors: learned

- Flexible goal-directed and habitual (aso goal directed)
- Relies on previous experiences
- Selects **actions that are associated with high rewards**
- **PFC and basal ganglia (BG)** = two complementary learning system (PFC slow but precise and abstract, stiatum = fast but prone to mistakes)
- Basal ganglia: the caudate, the putamen, the globus pallidus, the substantia nigra, and the subthalamic nucleus
- **Dopamine** from VTA and SNpc offers a **training signal to 'tag'** rewarded actions
- **'reward prediction error'**
- Dopamine strenghtens synapses, activation of which is followed by reward, and weakens synapses, activation of which leads to 'negative prediction error'
- Both striatum (part of BG) and PFC are innervated by dopamine
- However, stritum is more densely innervated with dopamine = allows for faster learning
- PFC, on the other hand, is less innervated with dopamine and learning occurs slower = allows **learning to be integrated across more experiences** - less chance for error, construction of more generalized representations
- **Generalized representations are essential when deciding in unfamiliar situations**

Innate vs. learned behaviors: learned

- Complex tasks can be imagined as a decision tree
- At each level one can choose among several responses
- At the end, the task is completed and results in a reward
- (it is hypothesized that) **flexible structure of PFC can capture entire tree structure** - forming an internal model of the task
- In complex task the reward is delayed
- **BG**, on the other hand, **learns only most rewarding alternative at each decision point**
- BG learning is fast, but inflexible
- Complex tasks require PFC, simple association tasks require only BG
- Inhibition of PFC by transcranial magnetic stimulation disrupts ability to use complex models to guide behavior and subjects select immediately rewarding option instead

Innate vs. learned behaviors: **flexible behavior and habit formation**

- If the required behavior to achive goal needs to remain flexible or the goal often changes behavior remains dependent on PFC

- However, if required behavior (even complex one) is unchanged, the sequence of appropriate actions to reach a goal becames dependent only on BG - forming a habit

- Inactivating BG disrupts well-learned behaviors

- However reccurent connections between BG and PFC exist (information in one is avaliable of the other)
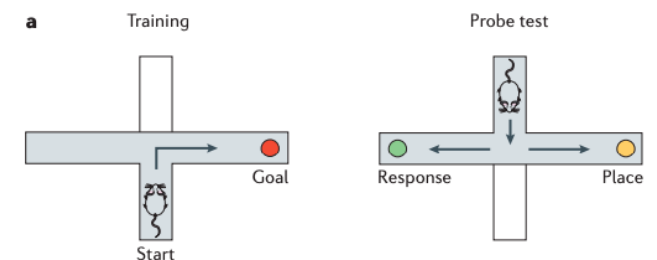
Habit formation - basal ganglia anatomy

- **D1DR-expressing MSNs** predominantly send inhibitory projections directly to the output nucleus of the basal ganglia: the globus pallidus interna/substantia nigra pars reticulata (GPi/SNr). This is referred to as the 'direct pathway' or 'D1 pathway'.

- **D2DR-expressing MSNs** predominantly send inhibitory projections first to the globus pallidus externa (GPe).The GPe then sends inhibitory projections to the subthalamic nucleus (STN). **The STN then sends excitatory projections back to all structures in the basal ganglia, including the GPi/SNR.** Consequently, this pathway is referred to as the 'indirect pathway' or 'D2 pathway'
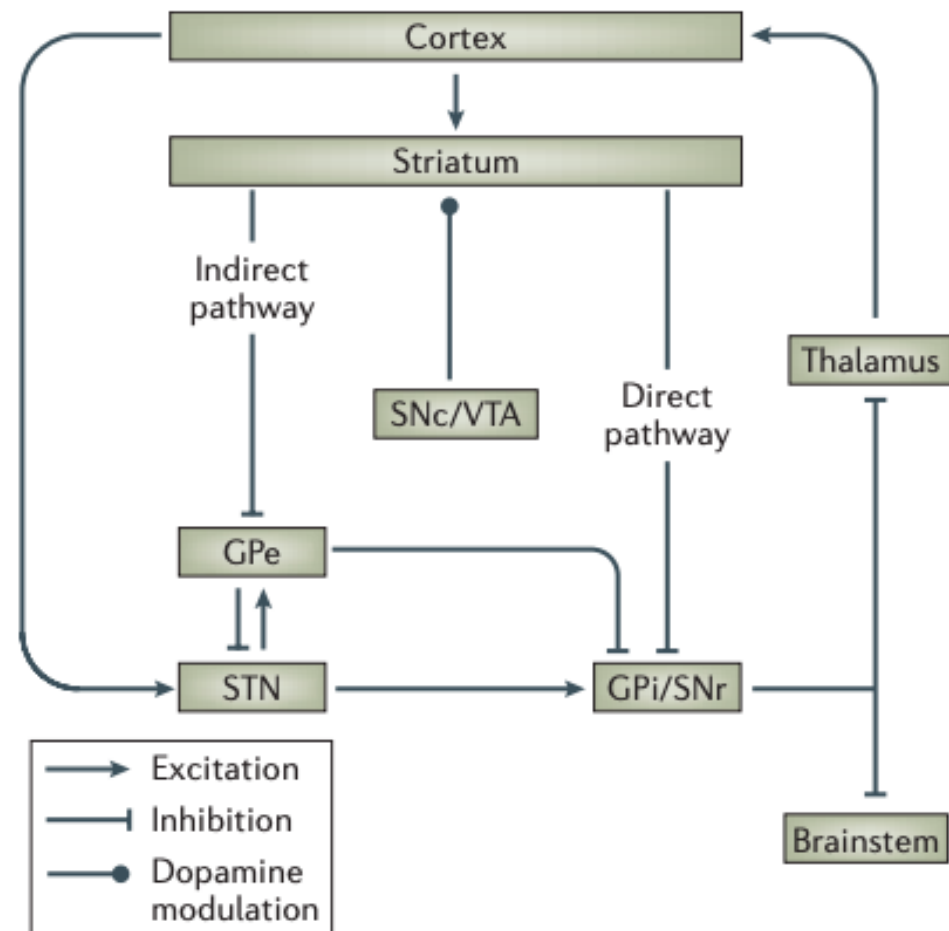
Habit formation - GPe feedback loop

- GPe neurons also project back to the striatum innervating both interneurons and MSN's neurons. The interneurons extend laterally across the parallel projecting pathways. Consequently, activity in individual D2 pathway may be used in lateral competition processes between competing pathways.

- This is in contrast to the simpler parallel pathway structure of the D1 system.

- D2 system likely developed later in evolution, refining response selection mechanism

Habit formation - role of dopamine

- D2 receptors are more sensitive dopamine therefore are always active - non-stop inhibition

- D1 receptors are less sensitive to dopamine, therefore higher dopamine level is needed to activate them

- **Prepare and select model** (PAS; Keeler et al., 2014)
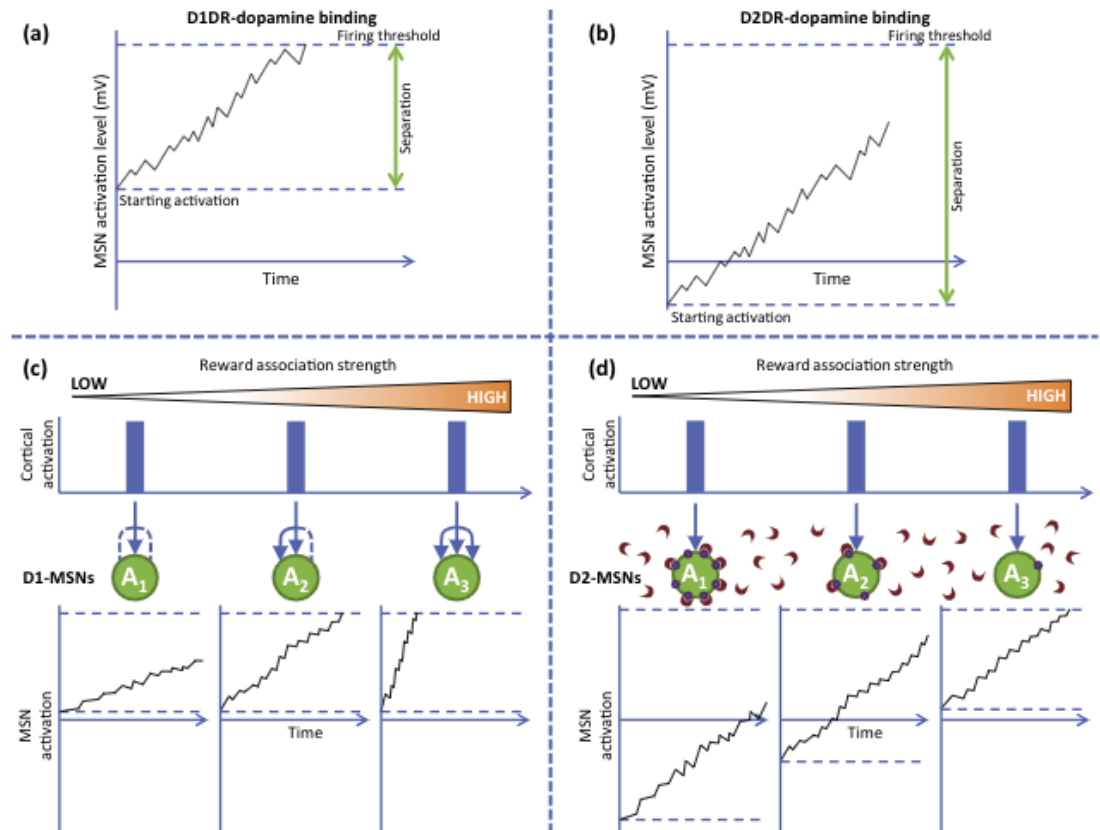
Parkinson's disease and Huntington's disease

- Parkinson - degeneration of SNc dopaminergic neurons
- Low dopamine leads to defect in movement initiation (threshold for activating direct pathway is not reached)
- Hyperactivity of GPe and hypoactivity of STN
- *Awakenings* by Oliver Sacks describes first use of levodopa in Parkinsonism after 1915 to 1926 epidemic of encephalitis lethargica

- Huntington's disease is characterized by degeneration of caudate and putamen (striatum)
- Causes involuntary movements
- https://www.youtube.com/watch?v=mkGfxi23Zv0

Stereotypical behavior

- Overuse of habit
- **Obsessive compulsive disorder (OCD)**, but also autism, schizophrenia, Tourette syndrome (but in TS stereotypical behaviors are simpler motor stereotypies)
- Hyperactvity within basal ganglia circuits
- An inactivation or a lesion of any part of the BG helps OCD symptoms
- SSRIs, SSRIs + antipsychotics
- benzodiazepines do not help –differential diagnosis
- OCD: stereotypical behaviors usually related to security (checking, washing hands)
- Movies: Aviator (2004), As good as it gets (1997)
- Modeling stereotypical behavior in rodents : D2/D3 agonist quinpirole

Stereotypical behavior
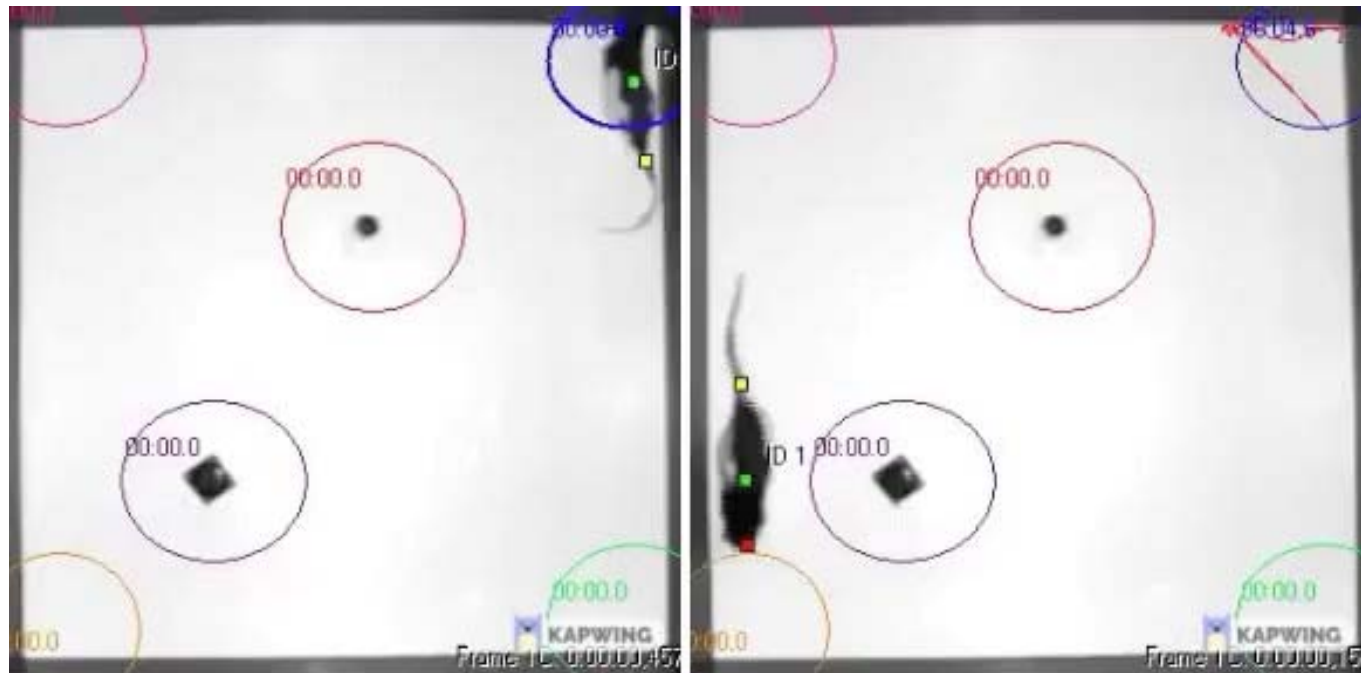
Innate behaviors: **Feeding**

- Nutrient intake is essential and requires food seeking and consumption behaviors
- There is a evolutionary pressure on feeding behavior and it is expected to be 'hard-wired'
- **Hypothalamus**: patients with hypothalamic injuries/tumors displayed rapid onset obesity
- In animals, damage to ventro-medial hypothalamus (VMH) and paraventricular nucleus (PVN) led to obesity
- In animals, damage to lateral hypothalamus (LH) led to anorexia
- VMH/PVN = 'satiety centre'; LH = 'hunger centre'

Feeding - external signals

- **Leptin** (released from adipose tissue)
- Ghrelin (released from empty stomach)
- Glucose
- Insulin
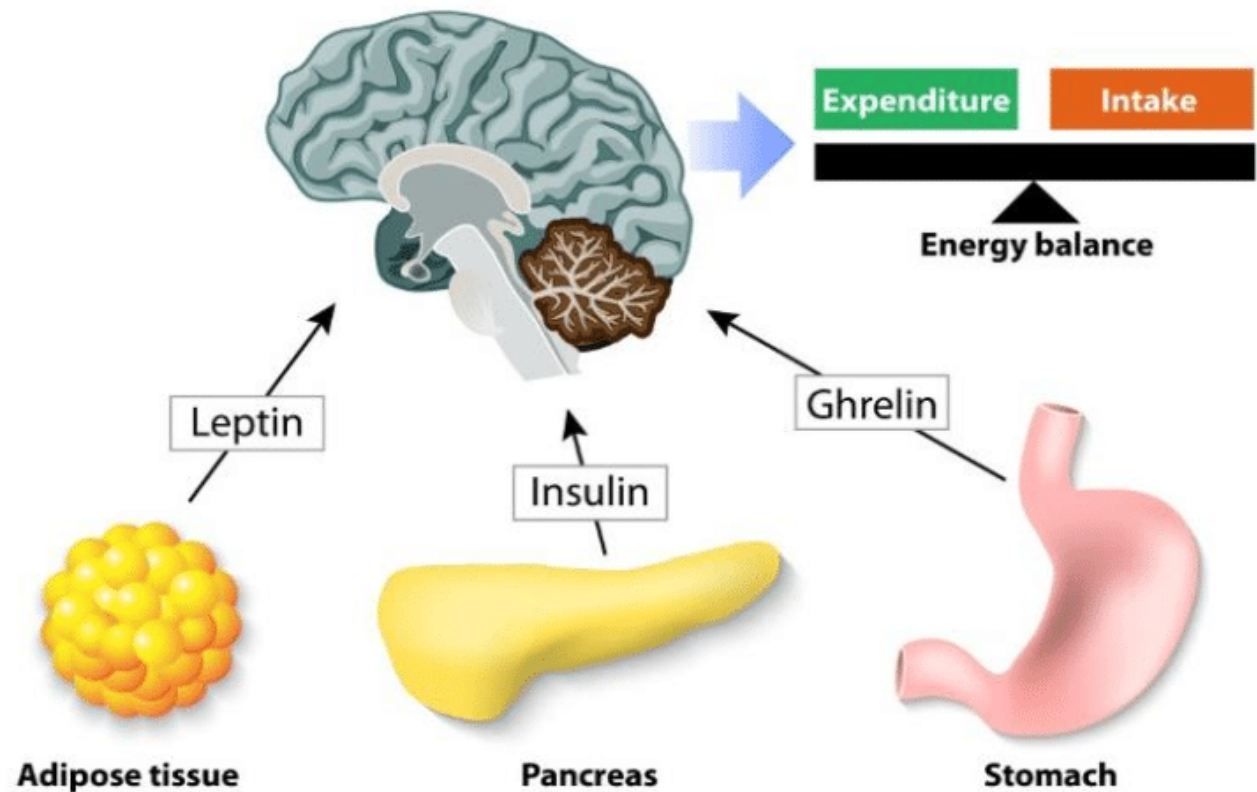
# Feeding – Agrp and α-MCH neurons of Arcuate nucleus

- <u>Arcuate nucleus</u> - leptin receptors (but aslo ghrelin, glucose and isulin receptors)
- Two groups of neurons: one group releases **Agouti related peptide (Agrp)** and **other Melanin-concentrating hormone alpha (α-MCH)**

- **On Agrp neurons leptin has inhibitory efect** – without leptin these neurons mediate hunger
- Smell of food also activated Agrp neurons - this means that Agrp neurons are also under neuronal control
- **Agrp neurons are inhibitory and release neuropeptide Y and GABA**
- Acute genetic ablation of Agrp neurons leads to starvation
- Optogenetic inhibition of Agrp neurons supresses feeding in starved animals
- Optognetic activation induces food foraging in satiated animals

- **On α-MCH neurons leptin exerts excitatory effects** - with leptin these neurons mediate satiety
- α-MCH neurons releases α-MCH, which activates melanocortin receptors and supresses feeding

Feeding – Agrp and α-MCH neurons of Arcuate nucleus
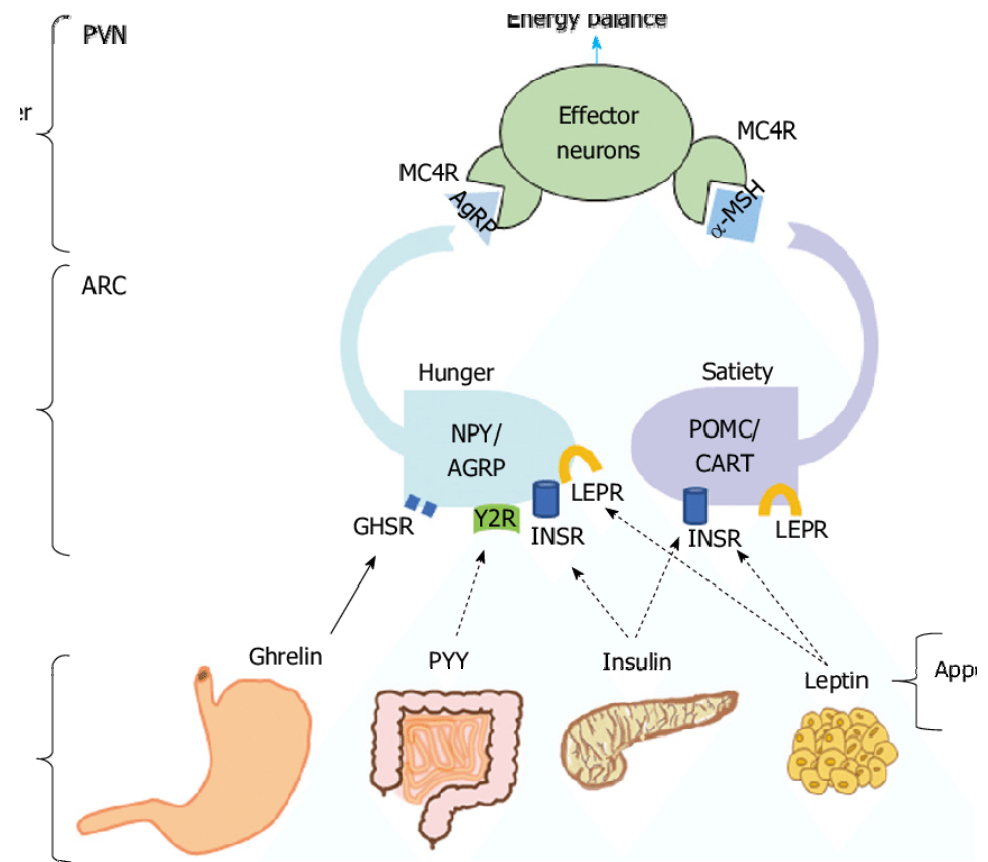
Feeding – Agrp neurons and their downstream targets

- Agrp neurons project to **paraventricular nucleus of hypothalamus** (PVN), orexigenic neurons in LH (lateral hypothalamic area LHA) and locally to α-MCH neurons of Arcuate nucleus

- to parabrachial nucleus (BPN)

- VMH/PVN = 'satiety centre'; LH = 'hunger centre'
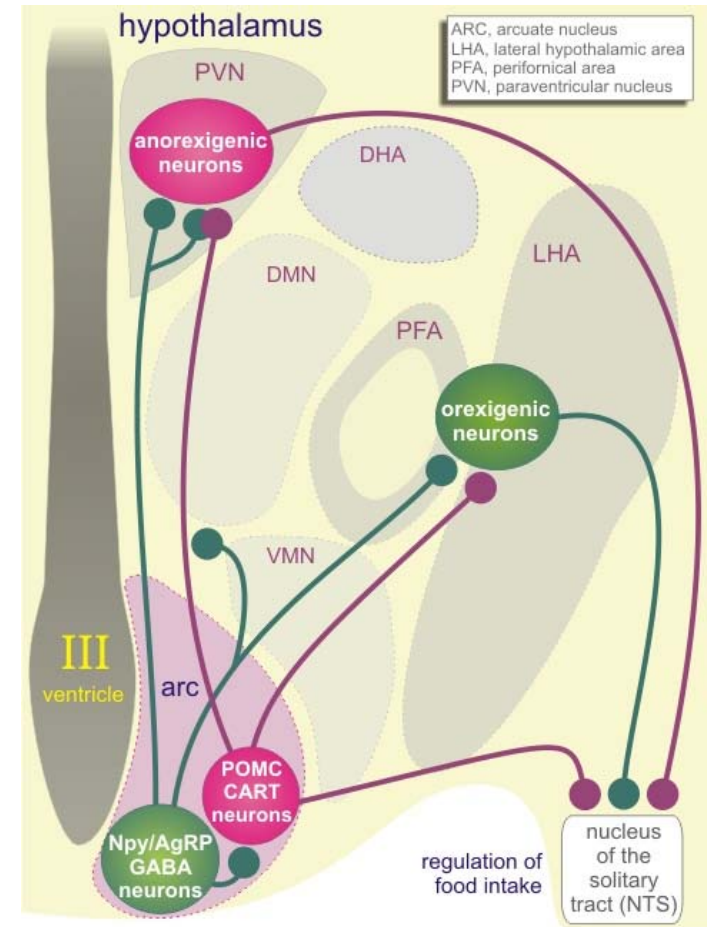
Feeding – hunger circuit

- Agrp neurons project to PVN
- **Activity of axons from Agrp neurons inhibits PVN** (anorexia centre)
- Othewise, PVN neurons releases oxytocin and inhibit nucleus of the solitary tract (NST) and inhibits food foraging responses
- NST integrates information from PVN and information about energetic and nutrient state from circulation (L-leucine, glucose, CCK, etc.)

Feeding – anorexia circuit

- Agrp neurons inhibits para-brachial nucleus (PBN)
- PBN receives visceral and taste information from the periphery (via nucleus of the solitary tract (NTS))
- PBN signals malaise and illness of GIT
- When Agrp neurons are ablated, cFos expression in PBN is elevated. (and animal feels sick)
- Injection of benzodiazepines into PBN rescues feeding behavior
- Therefore, **PBN actively supresses feeding behavior**, but during hunger it is supressed by Arcuate nucleus
- In intact mice **ablating BPN increases feeding behavior**

Aggressivity

- innate behavior with the purpose to protect and ensure societal status
- regulated by environmental, hormonal, and experiential factors
- Observed mostly in males except for lactating females
- **Resident-intruder test**
- Maternal aggression
  - Hormonal changes and exteroceptive stimulation by pups
- Male aggressivity towards pups
  - Virgin males
- Intermale agressivity
  - Follows a stereotyped escalating pattern until one combatant assumes a submissive position
  - Serves to establish interindividual hierarchy
  - Persitence upon removal of the stimulus – hysteresis
  - Associated with rewarding properties
- Submissive behavior
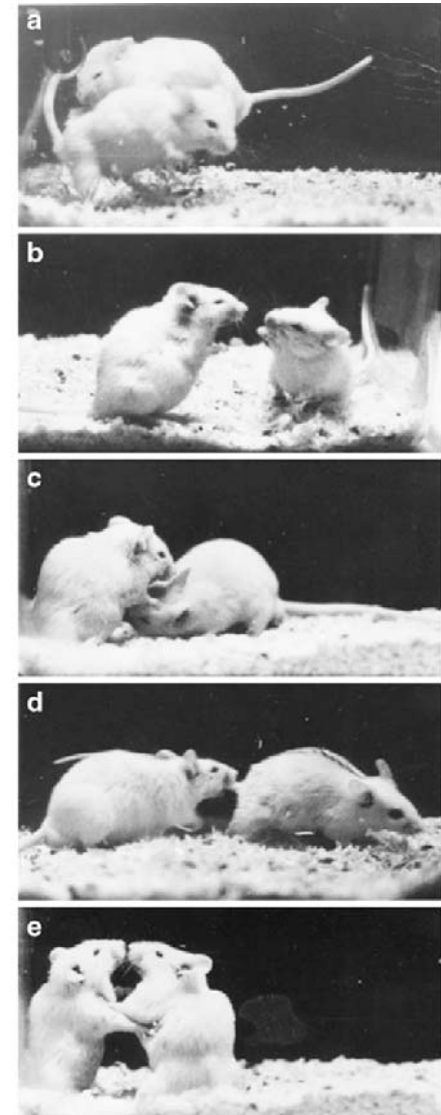
Aggressivity - main agressivity hub: MEA-PMv-VMHvl

- **Medial amygdala (MEA)** recieves olfactory input (raleys info to **ventral premammalary nucleus (PMv)** and **hypothalamic agression area(HAA))**

- Optogenetic activation of PMv triggers attack, optogenetic silencing PMv terminates attack

- Projects to HAA (ventrolateral part of the ventromedial hypothalamus: VMHvl)

- optogenetic activation of VMHvl neurons induces immediate attacks in male mice, while chemogenetic inhibition of VMHvl neurons decreases normal aggression

- **Both PMv and VMHvl can drive agression without sensory input**

- In males, only optogenetic activation of VMHvl neurons that express estrogen receptor alpha trigggers attack. In females opto activation of same neurons do not induce agression. Estrogen receptor alpha is a transcription factor.

- Highlights importantce of sex hormones in aggressive behavior and intersex differences in expression of agressivity

Aggressivity - outputs and inputs from the main agressivity hub

- VMHvl projections to **bed of stria terminalis (BSTv)**
- **BSTv activates periaqueductal gray** (PAG; motoric response) and **PVN** (humoral response)
- **Upstream regulator of MEA is likely posterior amygdala (PA) that recieves input from ventral hippocampus and vomeronasal organ**
- PA processes input and relays signal to MEA, VMHvl (in case of agression, glutamate releasing neurons).
- Again, PA neurons that process this information highly express estrogen receptor alpha
- During lactation estrogen levels are low, probably contributing to maternal agressivity during lactation

Aggressivity - male behavior towards females and males

- 2 population of 'sex-specific' neurons within VMHvl
- Population active with male conspecific triggers aggression
- Population active with female conspecific triggers sexual behavior
- Thought to be hardwired, but…
- Isolated male does not respond to new male with an aggressive response
- And populations of these neurons are not separated during first exposures to males and females
- These populations separate after repeated exposition to female
- Aggressive and sexual behavior is very related
- Hypothesized that humans with aggressive sexual behaviors have neuronal hardwiring problem in VMHvl
- We see that in animal facilities: male can live with males, but once mated, canot be reintroduce into male cage

Parental care - main characteristics

- Behavior directed towards immature conspecifics that improves a probability of their survival
- Most developed in mammals and birds
- Retrieval, crouching, licking and nestbuilding (and maternal aggression)
- Hormone dependent: virgin females usually ignore pups but will display maternal behavior if they are in close contact with pups or are hormonally stimulated
- Males usually attack pups but will show parental care at the time after mating when their pups are supposed to be born
- Antagonistic pathway to aggression
- **medial preoptic area (mPOA) of hypothalamus**

Parental care - mPOA

- medial preoptic area (mPOA) of hypothalamus
- **Extent of mPOA activation correlates with the quality of parental care**
- **Lesion of mPOA abolishes parental care**
- Hormones can act directly via mPOA: infusing estrogen or prolactin into the mPOA of virgin female rate hastens the onset of maternal care
- mPOA inhibits defensive/aggressive behaviors via **inhibiting VMHvl**
- Similarly to VMHvl recieves input from medial amygdala (MEA)
- In virgin males signal from pups activates MEA – VMHvl pathway leading to male agressivity towards pups
- In virgin males lesion of MEA and vomeronasal organ decreases agressivity and promotes parental care

Parental care - galanin neurons in mPOA

- Recently it was shown that galanin expressing mPOA neurons are responsible for parental care - selectively inhibiting galanin expressing neurons impairs all components of parental care

- Optogenetic activation of galanin expressing mPOA neurons induces pup grooming in male virgin mice (and decreases agression towards pups)

- However, activation of galanin neurons fails to evoke other components of parental behaviors such as retrieval and nestbuilding

Parental care - mPOA and dopamine

- **mPOA projets to VTA** - probably reinforcement plays a role in parental behavior

- Inhibition of VTA disrupts components of maternal behavior

- Dopamine signalling is therefore important in parental care

Vocalication

- **Measurement of general emotional state of the animal**
- Measurement of social interactions
- Measurement of fear response

- Between rodents: **ultrasound (>22kHz)**
- Communication with oher species: audible (humans: 20 Hz to 20 kHz.)
- Ultrasound vocalization
- 50 species of rodents emit USV
- Frequency range 22kHz for aversive calls, 50kHz for positive calls

Vocalization - positive 50 kHz calls

50 kHz calls can be subdivided:

- **Flat 50kHz calls**
  - During social situations
  - During consumption or expectation of palatable food
- Frequency modulated 50 kHz calls ('**step calls**')
  - Strongly rewarded and highly motivated situations (eg. sexual situations)
- Frequency modulated **50kHz calls with trills**
  - Highest pleasure
  - Associated with self administration of cocaine
  - Reduces first during abstinence in addicted rats
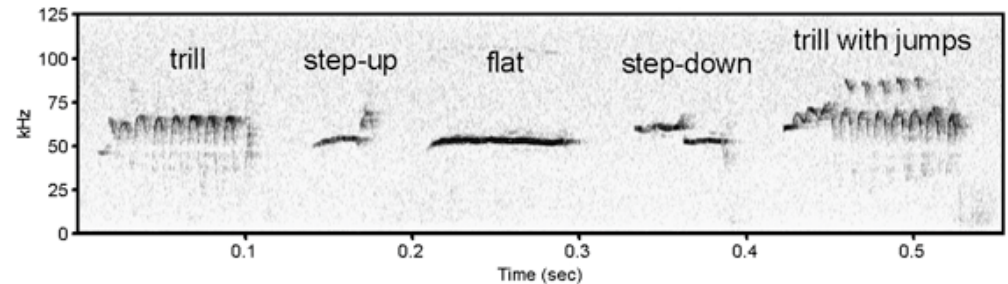
Vocalization - positive 50 kHz calls - examples

- Analogue of human laughter
- Juvenile play
- Tickling by the researcher
- Mating (when male is exposed to estrous female)
- Positive social encounters
- Replay of 50kHz calls
- Sucrose self administration or selection of sweet treats
- Electrical stimulation of nucleus acumbens, raphe, VTA or anticipation of therof
- Anticipation of alcohol self-administration
- In alcohol-dependent rats, number of emitted 50 kHz calls positively correlated with the amount of drunken alcohol
- 50kHz calls associated with release od dopamine from nucleus accumbens
- **Most 50kHz calls when amphetamine is injected directly into nucleus accumbens. So that is probably the best thing ever.**

Vocalization - negative 22 kHz calls

- Divided into **short** (less than 300ms) and **long** 22kHz calls (more than 300ms)

- Short 22 kHz calls: internal aversion

- Long 22 kHz calls: danger

- Choligenic stimulation – carbachol inudes vocalization of short 22kHz calls

- injection of glutamate into the laterodorsal tegmental nucleus

Vocalization - 22kHz aversive calls - examples

- Associated with aversive state
- Displeasure, anxiety, chronic fear, or dysphoria
- Chronic pain (attenuated by aspirin and morphine)
- Rats facing predators
- Attenuated by systemic morphine
- Foot shock, lound acoustic stimuli, unexpected airpuff
- Encounter with the dominant rat
- Defeated rats
- Close approach of unfamiliar human
- Prolonged isolation
- After ejaculation in males
- Withdrawal from addicitve agents (alcohol, benzodiazepines, stimulants, opiates)
- Decreased doses of cocaine

- Associated with decrease in their locomotor activity, increase in behavioural inhibition and freezing responses, erect body hair
- Events associated with 22kHz calls remain more stable in the memory
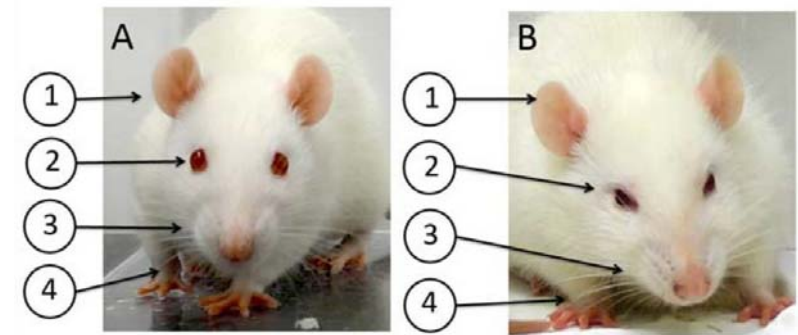
Rat Grimace Scale (RGS)
- Orbital Tightening: narrowing of the orbital area, partial or complete eye closure or squeezing
- Nose/Cheek Flattening: with eventual absence of the crease between the cheek and whisker pads
- Ear Changes : fold, curl and angle forwards or outwards, pointed shape
- Whisker Change: move forward away from face

Vocalization

- Why are rodents signalling their emotional state to their conspecifics?
- Hypothesized that evolved early due to maternal/paternal care of infants
- Infant distress calls are universal in mammalian kingdom
  - Mothers that were able to control pups from the distance were selected for
  - Pups that could not effctively communicate were eliminated
- Aversive calls are adaptive due to obvious advantage for the social group (signaling danger)
- Adaptive value of 50kHz calls is not that well established (but could be advantageous during singnalization of palatable food)

Vocalization – neuronal substrates

- Both initiated in **tegmentum** - both part of reticular ascending activating system
- Positive calls:
  - Initiation: mesolimbic dopamine system from V<u>T</u>A to ventral striatum
  - Electrical stimulation of VTA produces 50kHz calls
  - Alternatively positive calls can be initiated by stimulation of **preoptic area of the hypothalamus (POA)** (still dopamine dependent as 50kHz calls can be inhibited by administration of dopamine antagonists)
  - Positive arousal
- Negative calls:
  - Initiation: mesolimbic choligenic system from laterodorsal tegmental nucleus (LDTg) and travelling to the medial regions of the diencephalon, basal forebrain, and lateral septum
  - Glutamate stimulation of **laterodorsal tegmental nucleus (LDTg)** induced 22 kHz vocalizations
  - LDTg engaged in mechanisms of anxiety/fear and promotion of emotional arousal under adverse conditions

Generation of movement sequences

- All behavior is at the end movement
- Rarely movement happens in isolation
- Most often, behavior is a sequence of simple movements
- When one movement finishes only then the other should begin
- What are the neuronal substrates of learning and executing motor sequences?

- **Motor cortex** = learning of sequences, voluntary flexible movement, dexterity
  - When motor cortex is ablated animal can execute learned sequences (but not learn new ones)
- **Cerebellum** = execution of learned motor sequences

Generation of movement sequences

- Cerebellum
- Coordination of voluntary movement
- Trace eye-blink conditioning = associative learning
- And recently, learning motor sequences
- Khilkevich et al., 2018
- End of one motor sequence serves as a cue (feedback signal –FS) to commence next movement in the sequence
- The nature of the FS is not known (deep cerebellar nucleus neurons (DCN); thalamus or from proprioceptive information
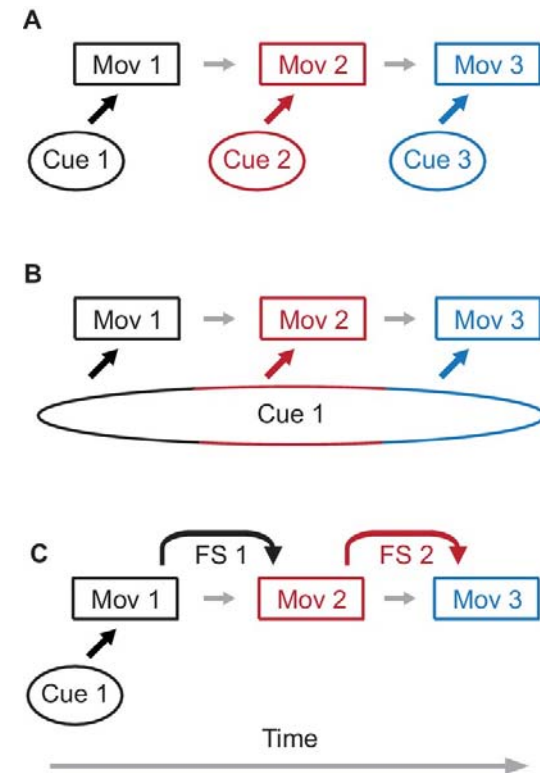
Thank you for your attention

- Any questions? Need links to original articles? Want join the team for PhD?
  - How are associations formed across a temporal gap?
  - What does cortical activity look like when you retrieve a memory?
  - Is there an energetic imbalance in the schizophrenia brain?
  - Do endocrine disruptors, such as BPA and BPS, lead to behavioral changes?
  - What are transcription markers of a memory?
  - Can we use neuroscience to improve forensic science?
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