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
  - Behavioral tools
  - Tools to modify neuronal activity/function
  - Tools to observe neuronal activity
- Pitfalls of current methods in behavioral neuroscience
- Innate behaviors
  - Feeding
  - Aggression
  - Parental behaviour
  - Vocalizations – signaling emotional state
- Goal directed (motivated) behaviors (Action-Outcome; Stimulus-Response)
- Habit fomation - basal ganglia anatomy and function
- Stereotypical behaviors

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 behaviour.
- Studies the interaction between the brain, body, environment and behaviour
- Behavioural vs cognitive neuroscience: behavioural pertains to movement, cognitive to mental processes

Tools to study neuronal basis of behavior

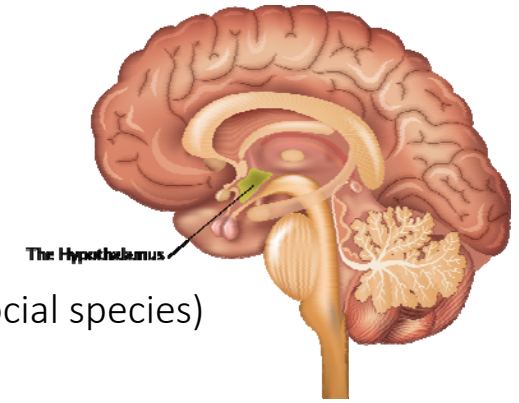
- Behavioral tools
  - Mazes
  - Operant tasks (reinforcement and punishment)
  - Observation of free movement
- Tools to interfere with normal brain function
  - Administration of agonist/antagonists (systemic, localized)
  - Lesions (permanent neuronal ablation)
  - Inactivation (temporary)
  - Optogenetics (increase decrease activity - localized)
  - Chemogenetics (increase decrease excitability – both localized and systemic)
  - Genetic models (knock outs, inducible knockouts (dox on dox off)
  - Transcranial magnetic stimulation (TMS)
- Tools to observe undisturbed brain activity
  - Immediate early genes
  - Electrophysiology
  - Calcium imaging
  - MRI, PET, EEG

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) = difficult to isolate a single process of interest
  - Usually only a single parameter is selected. If more parameters are selected usually inapproprate statistical methods are used (MANOVA = right; many separate 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 neuronal).
  - 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 administration of agonists/antagonist inactivations/facilitations of brain regional activity (muscimol, optogenetic, chemogenetic): 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
  - Limitations:
    - Electrophysiology: relatively small areas can be observed at the same time (but very good temporal and spatial 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 and spatial resolution in rodents - but you can record whole brain
    - PET, EEG - low spatial resolution, EEG - good temporal resolution

- TAKE HOME: do not trust every experiment that you read about

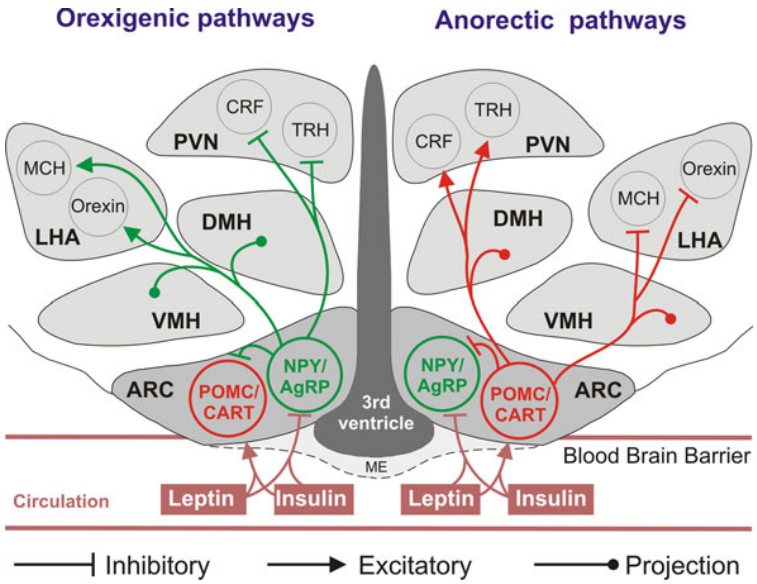
Innate behaviors



- Innate behaviors do not require learning (feeding, defence, parental care, sociability in social species)
- '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
- Innate behaviors are complex
- Species-specific
- Hypothalamus is essential for expression of innate behaviors (four F's": fighting, fleeing, feeding, and mating)
  - Below thalamus - bottom of the brain
  - More than 20 nuclei
  - Integrates signals from periphery and from CNS
  - More permeable BBB than other brain regions
- Before advent of molecular techniques hypothalamus was difficult to study: nuclei are very interconected and each nuclei contains different types of neurons responsible for different functions- more selective methods avaliable in the last decade
- Common principles: integratory hub, redundancy and neuronal population with antagonistic function withing the same nucleus (recieve same inputs, project to same areas but use different neurotransmitter to convey opposite 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

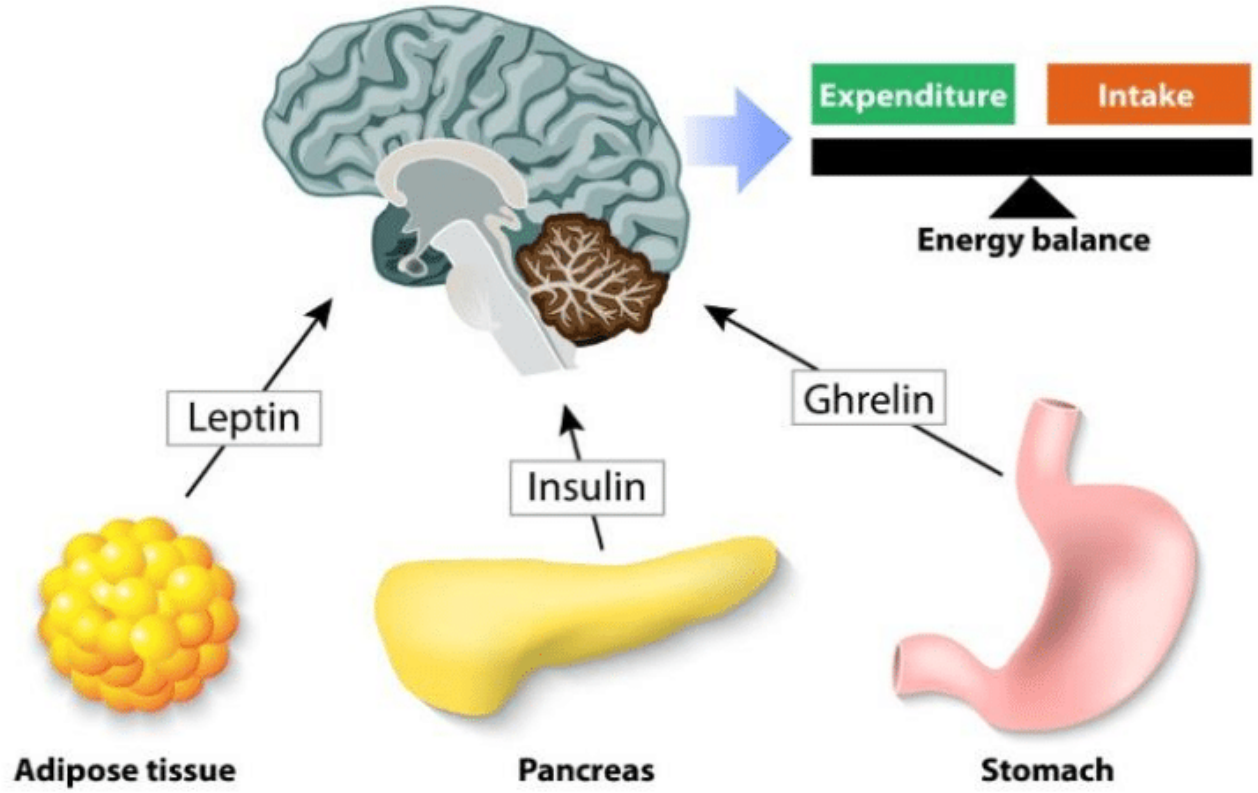
Feeding

- 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 VMH and PVN led to obesity
- VMH/PVN = 'satiety centres';
- In animals, damage to LH led to anorexia
- LH = 'hunger centre'



Feeding - external signals

- Leptin (adipose tissue)
- Ghrelin (released from empty stomach)
- Glucose
- Insulin

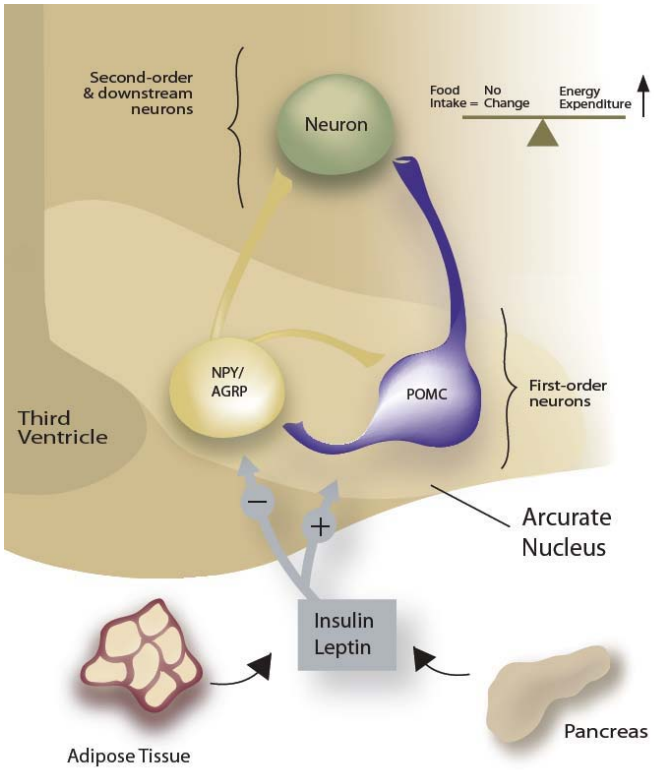


Feeding – Agrp and α-MCH neurons of Arcuate nucleus

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

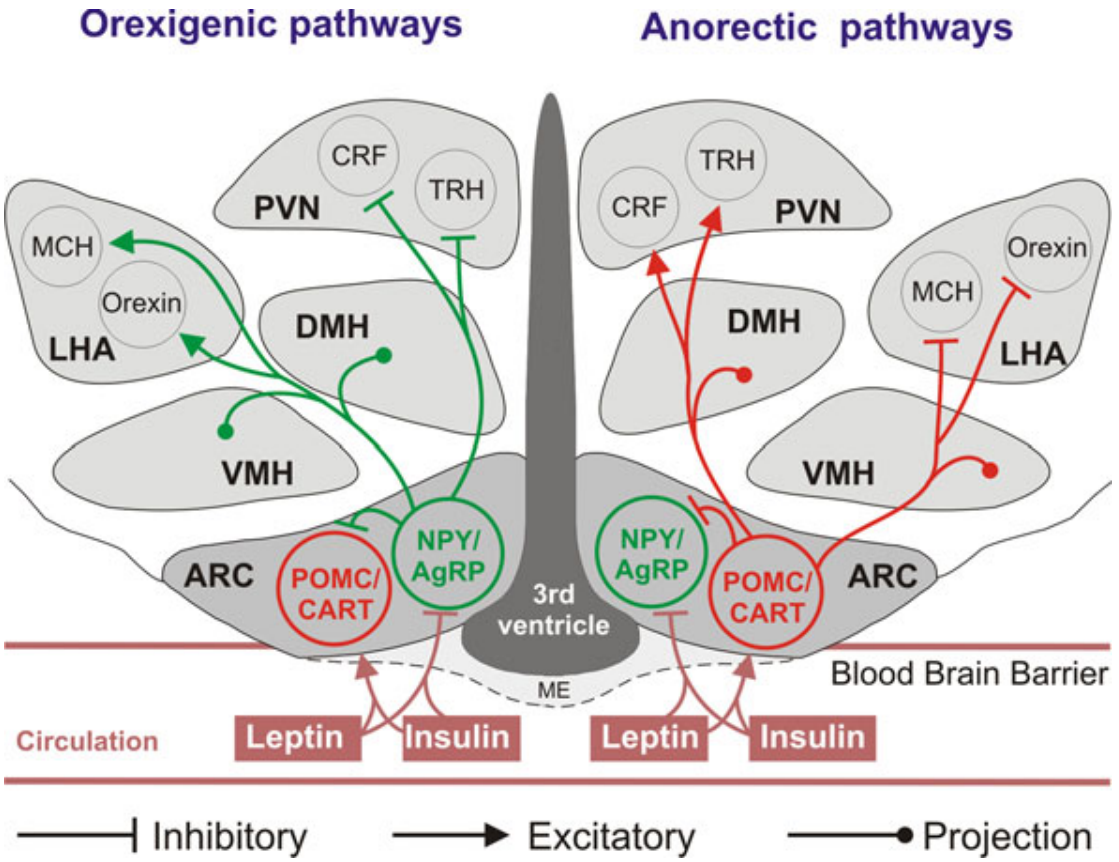
- No leptin → Agrp neurons active → consumption
  (leptin has inhibitory effect on Agrp neurons)
- Smell of food also activated Agrp neurons - this means that Agrp neurons are also under neuronal control
- Agrp neurons are inhibitory and release neuropeptide Y and GABA
- In adults optogenetic inhibition of Agrp neurons supressed feeding in starved animals
- Optognetic activation induces food foraging in satiated animals

- Leptin → α-MCH neurons active → satiety
  (leptin has excitatory effects α-MCH neurons)
- α-MCH, releases from Arcuate α-MCH neurons activates melanocortin receptors and supresses feeding



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
- LHA neurons release orexin. Lesion of LHA leads to starvation of animals
- And to parabrachial nucleus (BPN)

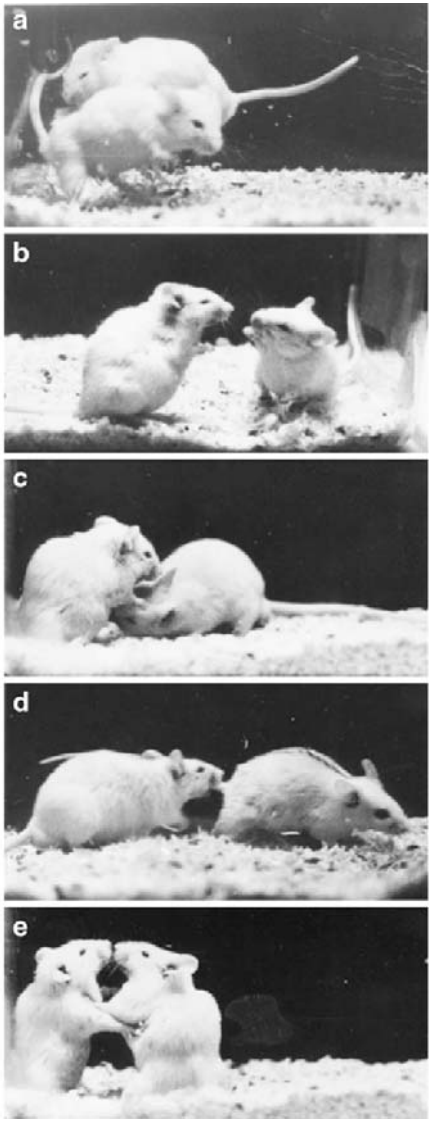


Feeding – anorexia circuit

- Normaly 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, secure resources and ensure societal status
- Regulated by environmental, hormonal, and experiential factors
- Observed mostly in males except for lactating females
- Resident-intruder test
- Intermale agressivity
  - Follows a stereotyped escalating pattern until one combatant assumes a submissive position
  - Serves to establish interindividual hierarchy
  - Perisitence upon removal of the stimulus – hysteresis
  - Associated with rewarding properties
- Maternal aggression
  - Hormonal changes and exteroceptive stimulation by pups
- Male aggressivity towards pups
  - Virgin males
- Submissive behavior



Aggressivity - main agressivity hub: MEA-PMv-VMHvl

- Medial amygdala (MEA) recieves olfactory input
- ventral premammillary nucleus (PMv) processes sensory information related to agression; only neurons that express dopamine transporter (but do not synthetize dopamine)
- Optogenetic activation of PMv triggers attack, optogenetic silencing PMv terminates attack
- Projects to ventromedial hypothalamus VMHvl
- optogenetic activation of VMHvl neurons induces immediate attacks in males, 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 PMv, VMHvl neurons that express estrogen receptor alpha triggers attack. In females opto activation of same neurons does 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

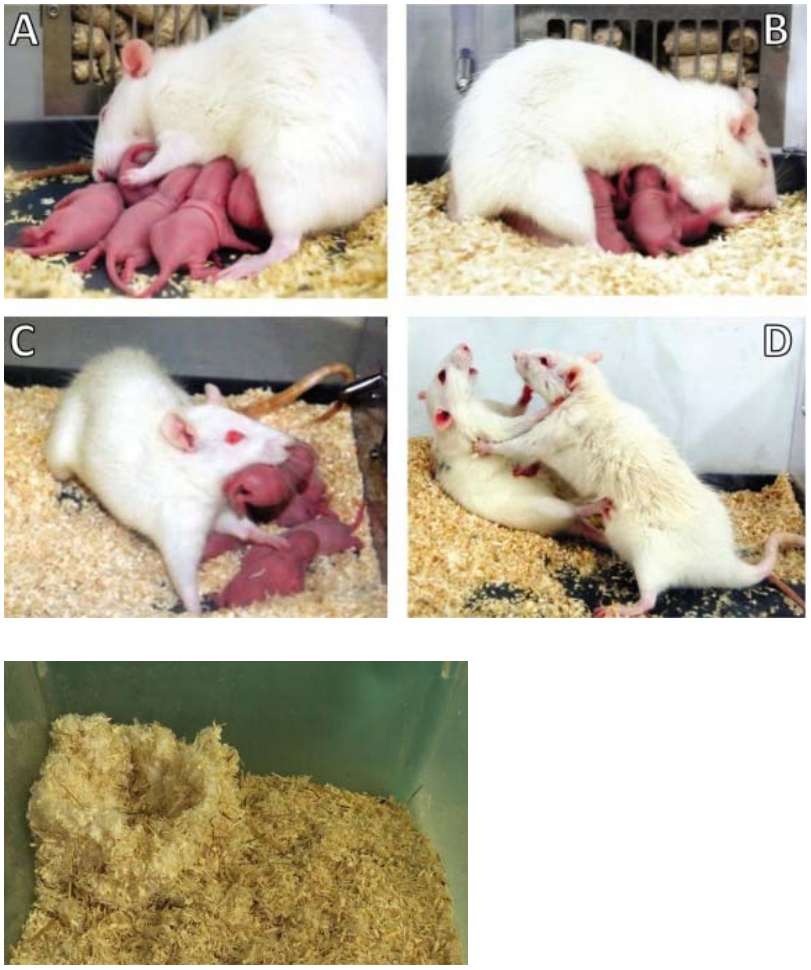
Aggressivity - inputs and outputs from the main agressivity hub

- Inputs:
  - posterior amygdala (PA) is an upstream regulator of MEA and recieves input from ventral hippocampus and vomeronasal organ
  - Vomeronasal organ is important in gender identification
  - Lesion of vomeronasal organ reduces aggressively
  - 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

- Outputs:
  - VMHvl projections to ventral portion of bed of stria terminalis (BSTv) – *luzkove jadro strie terminalis*
  - BSTv activates periaqueductal grey (PAG; motoric response) and PVN (humoric response : increase adrenaline, corticosterone)
  - PAG triggers stereotyped aggressive motor responses

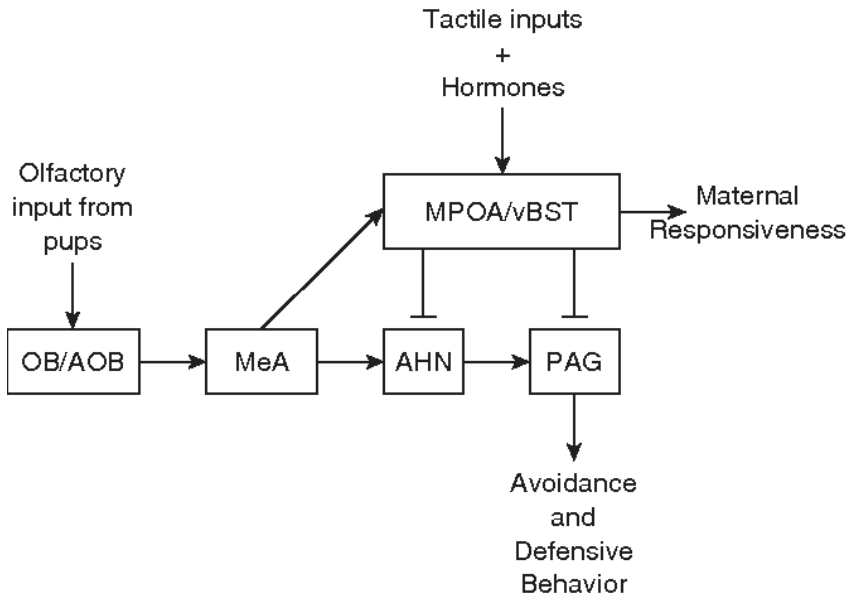
Parental care - main characteristics

- 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 oestrogen or prolactin into the mPOA of virgin female rate hastens the onset of maternal care
- mPOA inhibits defensive/aggressive behaviors via inhibiting VMH and AHN
- Similarly to VMH recieves input from medial amygdala (MEA)
- MEA recieves input from olfactory bulb (pup smell)
- 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 of virgin males and promotes parental care



Parental care - galanin neurons in mPOA

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

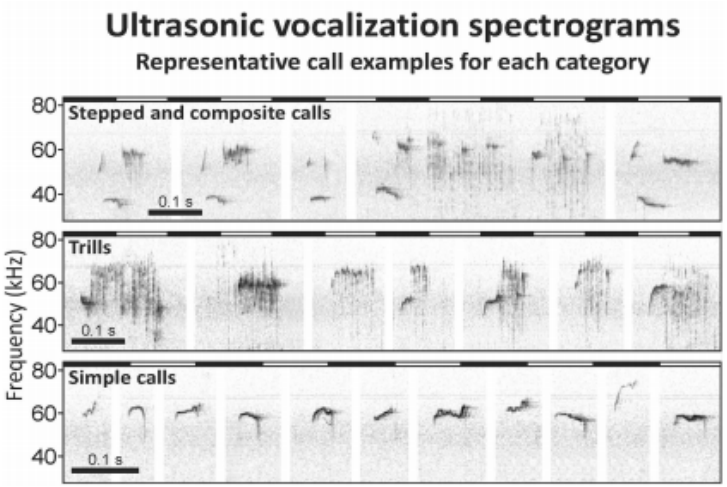
Vocalization

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

- Between rodents: ultrasound
- Communication with other 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

- Induced by activation of dopamine D_1, D_2 and D_3 receptors (all have to be activated concurrently)
- Analogue of human laughter
- 50 kHz calls can be further 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

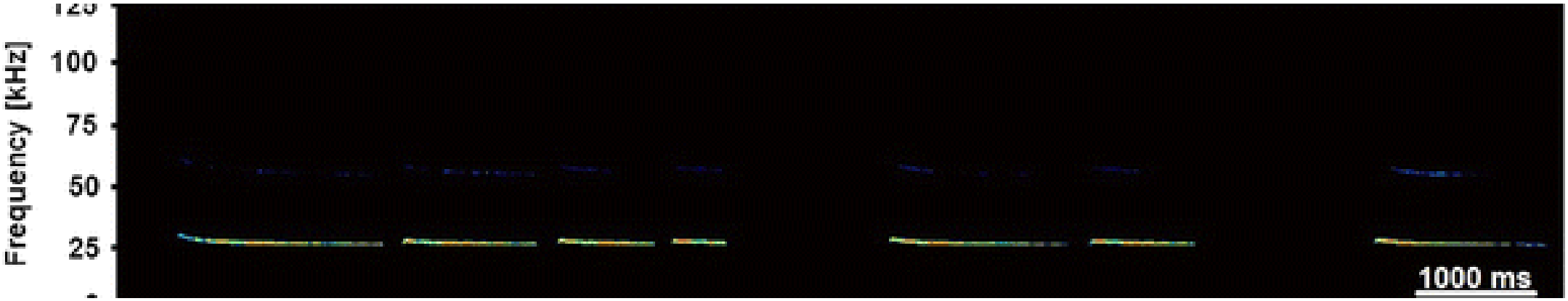


Vocalization - positive - examples

- Apetitive calls (50kHz)
- 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
- Anticipation of alcohol self-administration
- In alcohol-dependent rats, number of emitted 50 kHz calls positively correlate with the amount of drunken alcohol
- Supressed by aversive stimuli
- Electrical stimulation of nucleus acumbens, raphe, VTA or anticipation of therof
- 50kHz calls associated with release of dopamine from nucleus accumbens
- Most 50kHz calls when amphetamine is injected directly into nucleus accumbens

Vocalization - negative

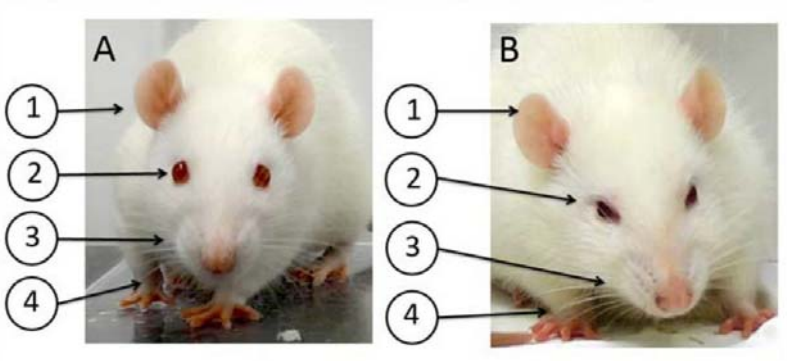
- Divided into short (less than 300ms) and long 22kHz calls (more than 300ms)
- Short 22 khz calls: internal aversion
- Long 22 kHz calls: danger
- Choligenic stimulation – carbachol inudes vocalization of short 22kHz calls
- injection of glutamate into the laterodorsal tegmental nucleus (choligenic)



Vocalization - negative examples

- 22kHz aversive calls
- 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

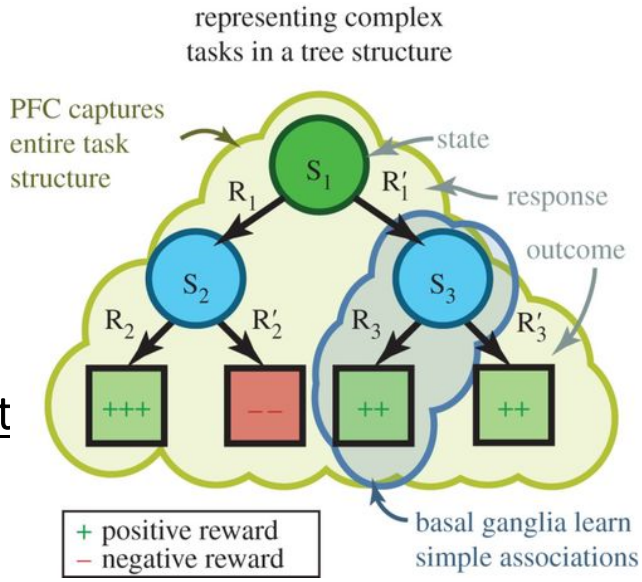
- Neuronal system responsible for initiation of vocalization
- Initiated in **tegmentum** - both part of reticular ascending activating system
- Positive calls:
  - Initiation: mesolimbic dopamine system from VTA to ventral striatum
  - Electrical stimulation of VTA produces 50kHz calls
  - Alternatively positive calls can be initiated by stimulation of hypothalamic-preoptic area (still dopamine dependent as 50kHz calls can be inhibited by administration of dopamine antagonists)
- Negative calls:
  - Initiation: mesolimbic choligenic signal from laterodorsal tegmental nucleus and travelling to the medial regions of the diencephalon, basal forebrain, and lateral septum
  - Glutamate stimulation of laterodorsal tegmental nucleus induced 22 kHz vocalizations

Learned behaviors

- Flexible-goal-directed or habitual (also are/were goal directed)
- Based on previous experiences
- Selects behavior that are associated with high rewards
- PFC and basal ganglia (BG) = two complementary learning system (PFC slow but precise and abstract, BG = fast but prone to mistakes)
- Basal ganglia: caudate (medial striatum), putamen (lateral striatum), and globus pallidus, the substantia nigra, and the subthalamic nucleus
- Dopamine from VTA and SNpc offers a training signal to 'tag' rewarded behaviors
- Dopamine strenghtens synapses associated with reward
- Absence of dopamine weakens synapses not assiciated by reward
- Both striatum (part of BG) and PFC are innervated by dopamine
- Striatum 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

Learned behaviors

- Many of the task are complex: composite of multiple steps to reach reward
- Complex tasks can be imagined as a decision tree
- At each level one can choose among several responses
- At the end, taks is completed and (hopefully) results in reward
- (it is hypothesized that) flexible structure of PFC can capture entire tree structure - forming an internal model of the task
- BG, on the other hand, learns only most rewarding alternative at each decision point
- Complex tasks require PFC, simple associating tasks 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

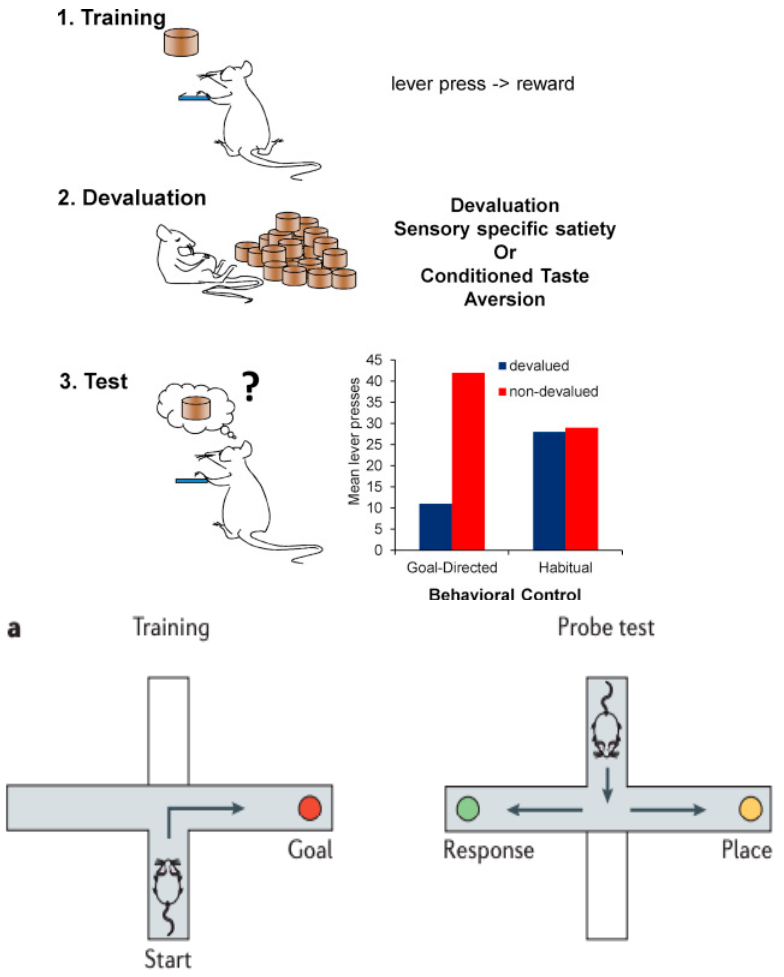


Flexible behavior and habit formation

- If the required behavior to achive goal needs to remain flexible or the goal often changes behavor 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
- In habits, topology of behavior is stabilized (only one way of many that behavior is done – way to work, putting on coat)

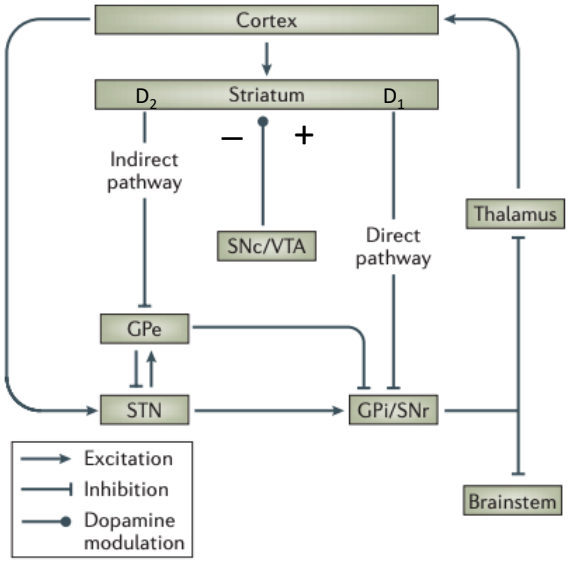
Behavioral tasks to study goal-directed and habitual behavior

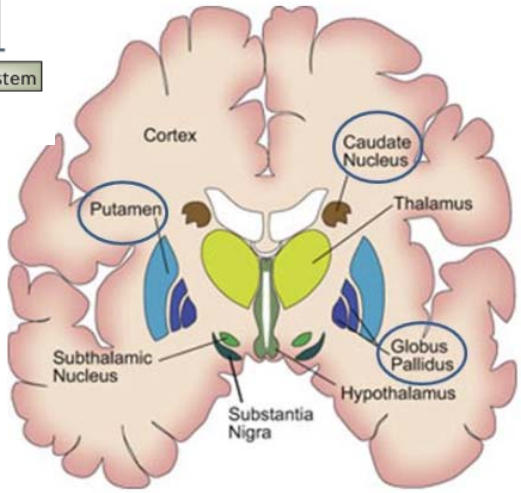
- Reward devaluation
  - Before testing animal receives abundance of reward
  - Only animals for which the action is habitual will respond
  - Animals that act in flexible goal directed manner will not respond because they do not care about reward that much at the moment
- Instrumental contingency
  - Random rewards are added to disrupt action-outcome contingency
  - Animals that rely on habitual responding are not sensitive to this manipulation an continue responding
  - Animals that behave in a flexible goal directed manner stop the action (as they can also get the reward for free)
- T-maze task
  - In a externa cue rich room animal is trained to turned to the same arm to receive reward
  - Test: animal is placed in the opposite arm and arm choice is recorded
- Sequential nose-poke task
  - To test composite tasks
  - To receive reward rat has to nose-poke a given sequence (eg. 3-2-5-4-6 → reward)



Habit formation - basal ganglia basic anatomy

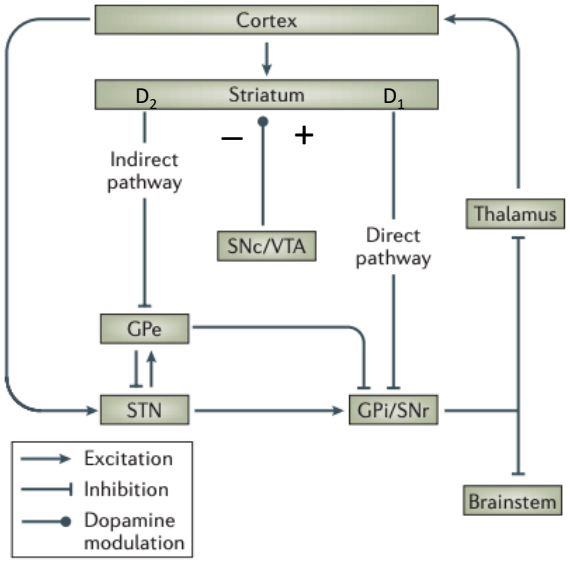
- Direct pathway (D1 pathway)
  - D1DR-expressing (medium spiny neurons) MSNs predominantly send inhibitory projections directly to the output nucleus of the basal ganglia: the globus pallidus interna/substantia nigra pars reticulata (GPi/SNr).
- Indirect pathway (D2 pathway)
  - D2DR-expressing MSNs 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.

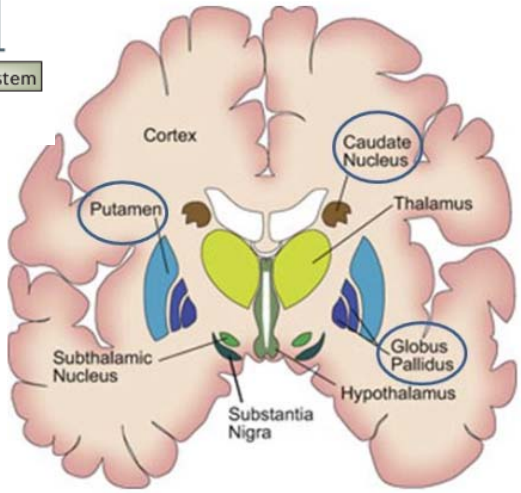




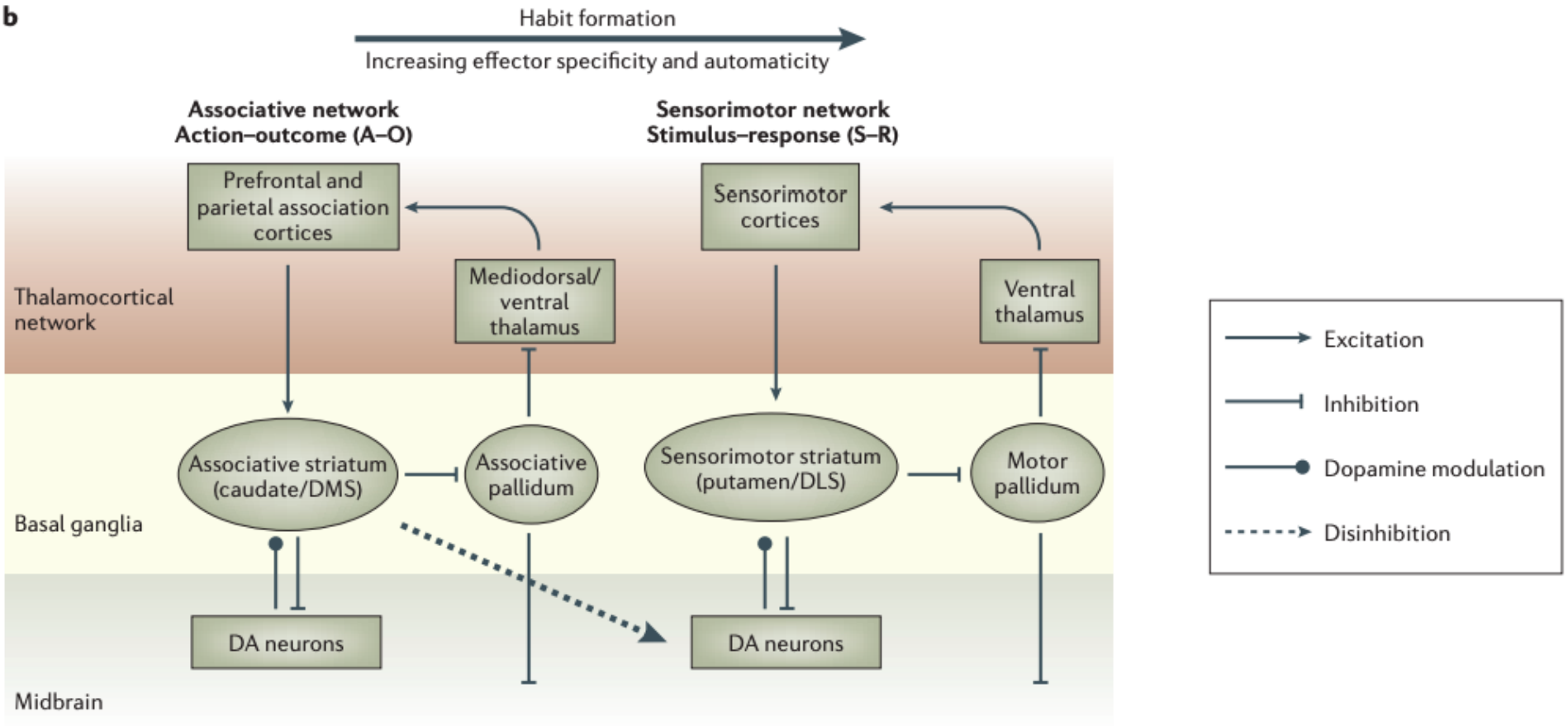
Habit formation - GPe feedback loop

- GPe neurons also project back to the striatum innervating both interneurons and MSN's neurons. The interneurons inhibit parallel projecting pathways.
- Consequently, activity in one D2 (indirect) pathway supress competing pathways.
- This contrasts with the simpler parallel pathway structure of the D1 (direct) pathway.
- D2 system likely developed later in evolution, refining response selection mechanism

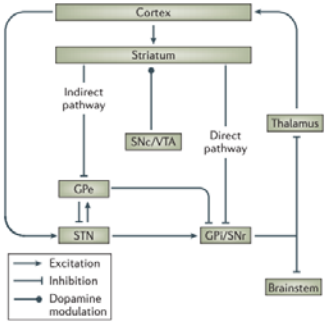




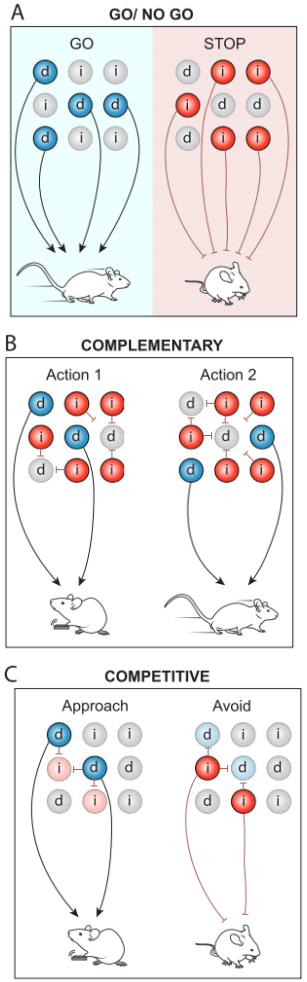
Habit formation - basal ganglia function



Habit formation - basal ganglia function

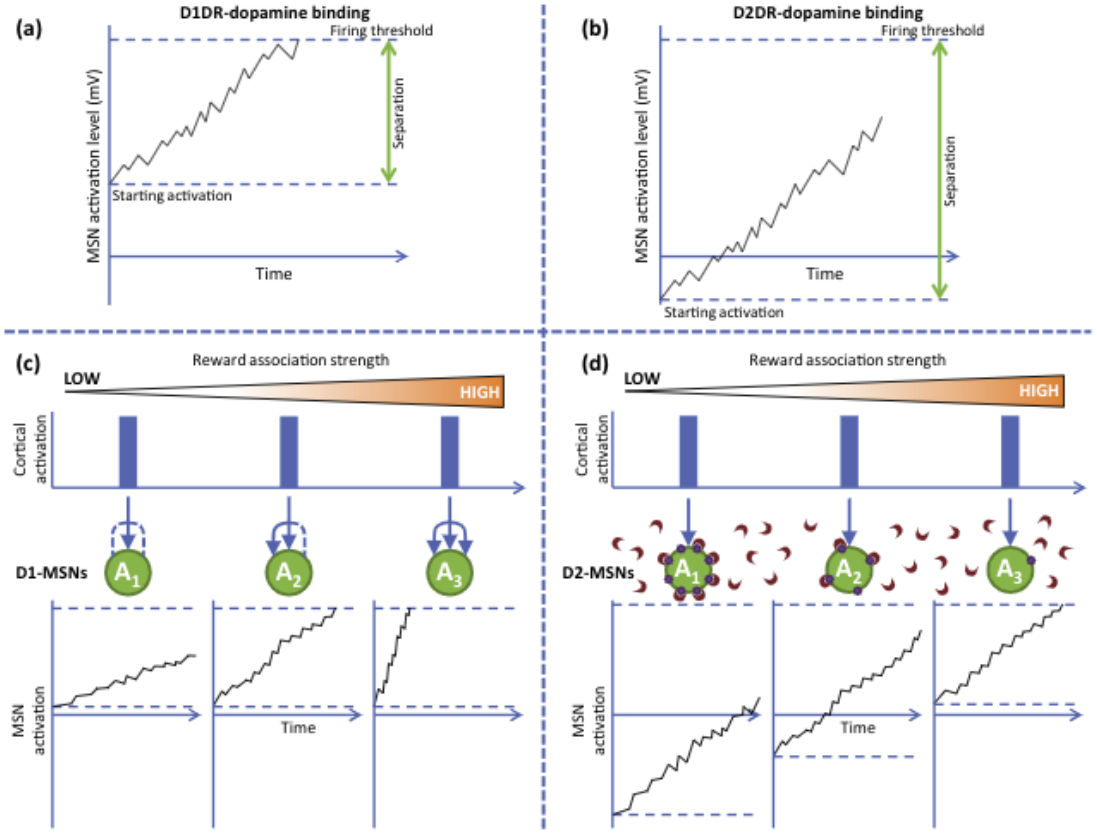


- Models of basal ganglia function
  - Go/no-go model
    - Stimulants increase movement
    - in Parkinson (low dopamine) patients display bradykinesia)
    - Ablation of iMSN results in hypoeractivity
    - Optogenetic activation fo iMSN inhibits movement
    - Optogenetic activation of dMSN facilitates movement
  - 'Complementary' model
    - Proposed, because recent evidence shown that iMSN are active during movement initiation
    - dMSN facilitate movement, while iMSN inhibit competing movements
    - 'inhibitory surround' seen in sensory systems such as the retina
  - 'Competitive' model (Bariselli et al., 2018)
    - Both iMSN and dMSN are tuned in to the same action, not conflicting action asi in complementary model
    - Indirect, iMSN, pathway mediates avoidance while direct, dMSN, pathway mediates approach
    - When iMSN and dMSN pathways are balanced subject hesitates (is undecided)
  - Indirect pathway provides a stop to selected action
  - Prepare and select model (PAS; Keeler et al., 2014)



Habit formation - prepare and select model

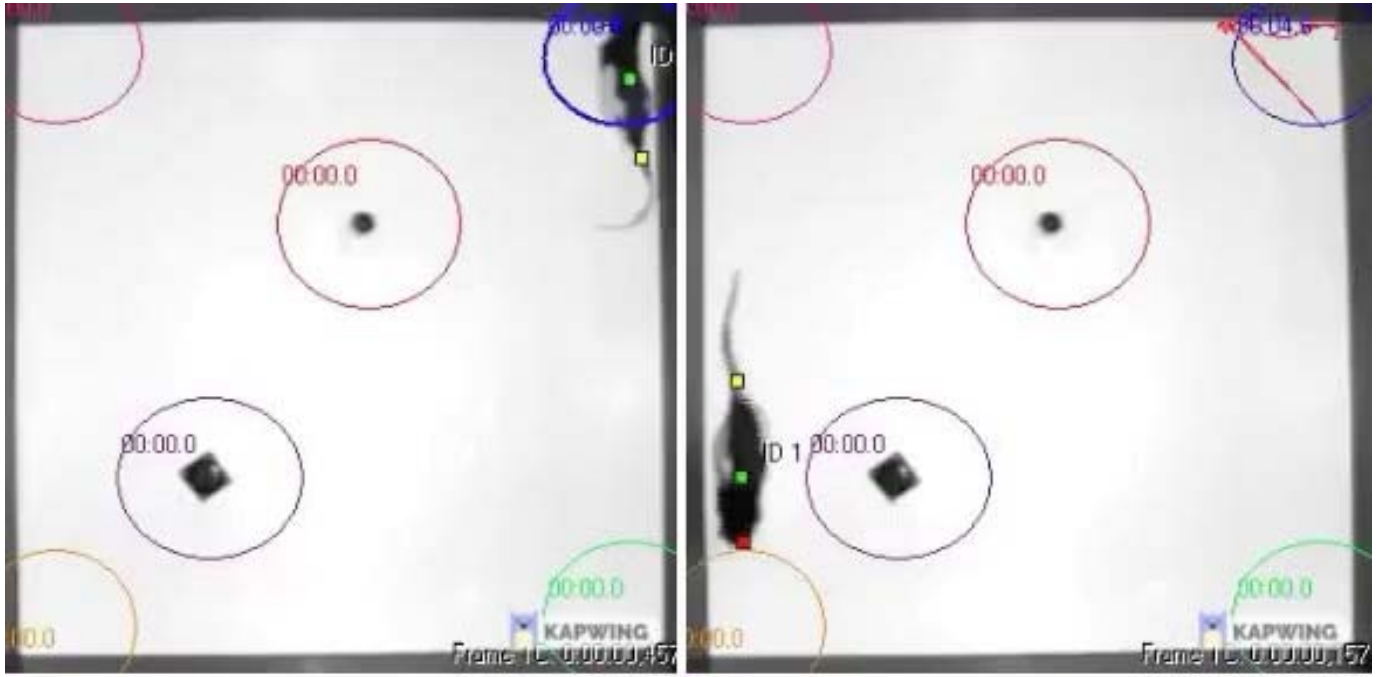
- Based on molecular evidence
- D2 receptors are more sensitive dopamine therefore are always active - non-stop inhibition
- D1 receptors are less sensitive to dopamine, therefore higher dopamine level is needed to activate them



Pathological stereotypical behavior

- Mal-adaptive use 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
- Psychotherapy, SSRIs, SSRIs + antipsychotics, benzodiazepines do not help – differential diagnosis
- Stimulation of STN (remember, that STN possibly inhibits recently selected actions)
- OCD: stereotypical behaviors usually related to security (checking, washing hands) – basic motivation
- Movies: Aviator (2004), As good as it gets (1997)
- Modeling stereotypical behavior in rodents : D_2/D_3 agonist quinpirole

Quinpirole induced stereotypical behavior in OF



Thank you for your attention