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
- Motivated behaviors (goal directed)
- Pavlovian associations
- Reinforcement
- Schedules of reinforcement
- Habit fomation
- Stereotypical behaviors
- Addictions
- Hypothalamus mediated behaviors: social approach, aggresivity, vocalizations

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

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

- Rodents: mouse, rat
- Behavioral tools
    - Morris water maze
    - Alothetic place avoidance
    - Sugar preference test
    - Forced swimming test
    - etc…
- 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
    - Engram tgging technology

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. If more parameters are selected usually inappropprate statistical methods are used (MANOVA = right; repeated 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 o when they are genetic model of geneticaly based disease (because persumably 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

- Basic division
- Innate
  - feeding, maternal behavior, sexual behavior, fear responses,…
  - Often depend on hypothalamus
- Learned
  - driving car, eating with utensils, reading,…
  - Basal ganglia, cortex

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, sociability 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- more selective methods avaliable in the last decade
- Common principles: integratory hub, redundancy and neuronal population with antoagonistic function withing the same nucleus (receive 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

Innate vs. learned behaviors: learned

- Flexible goal-directed and habitual 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: caudate, putamen, and 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

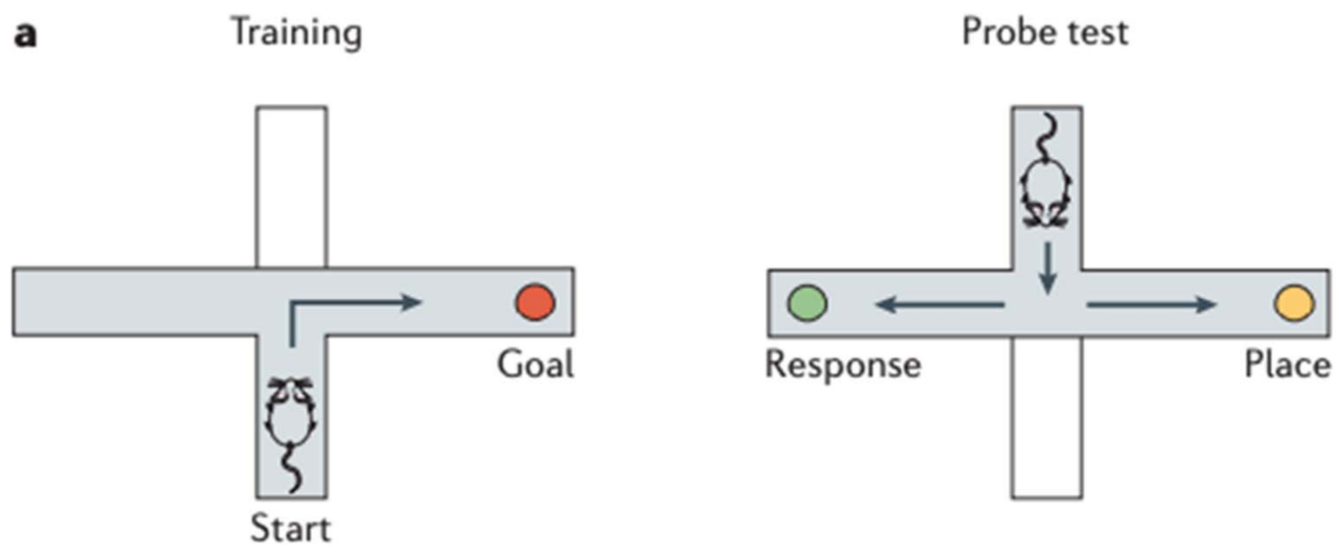
Innate vs. learned behaviors: learned

- Complex tasks can be imagined as a decision tree
- At each level one can choose among several responses
- At the end, taks is completed and results in 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 associateion 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

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 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
- However reccurent connections between BG and PFC exist (information in one is avaliable of the other)

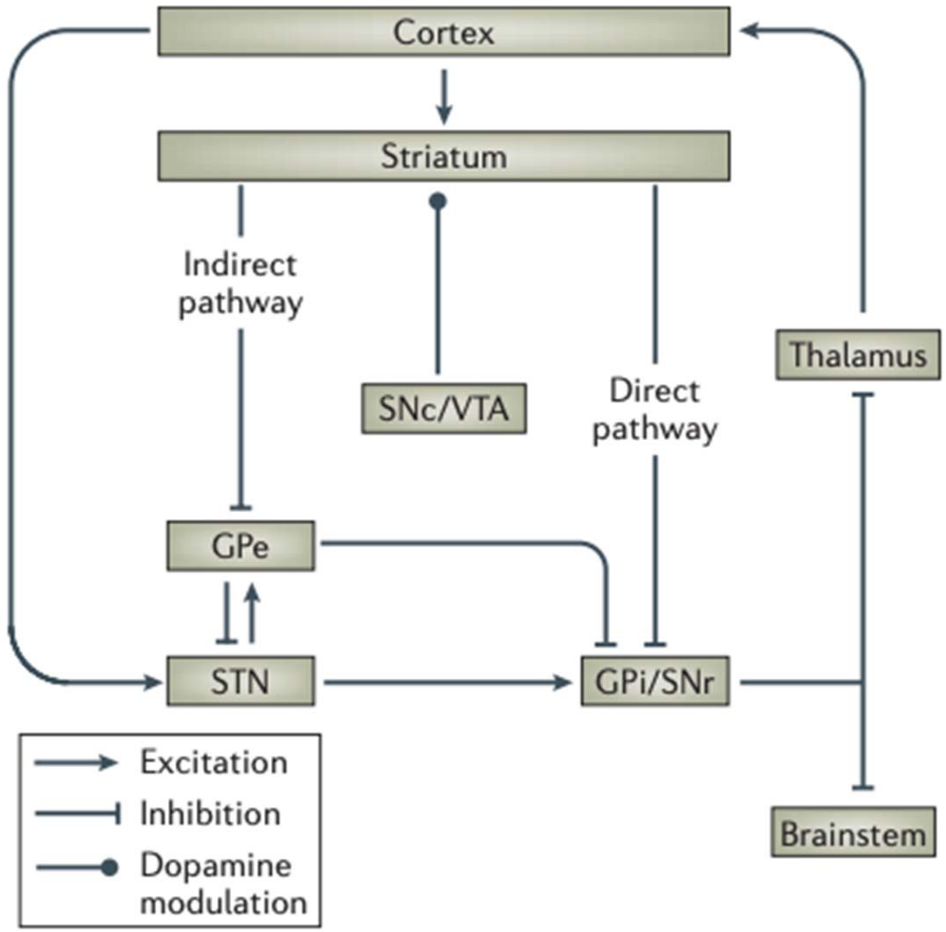
Flexible or habit? PFC or BG?



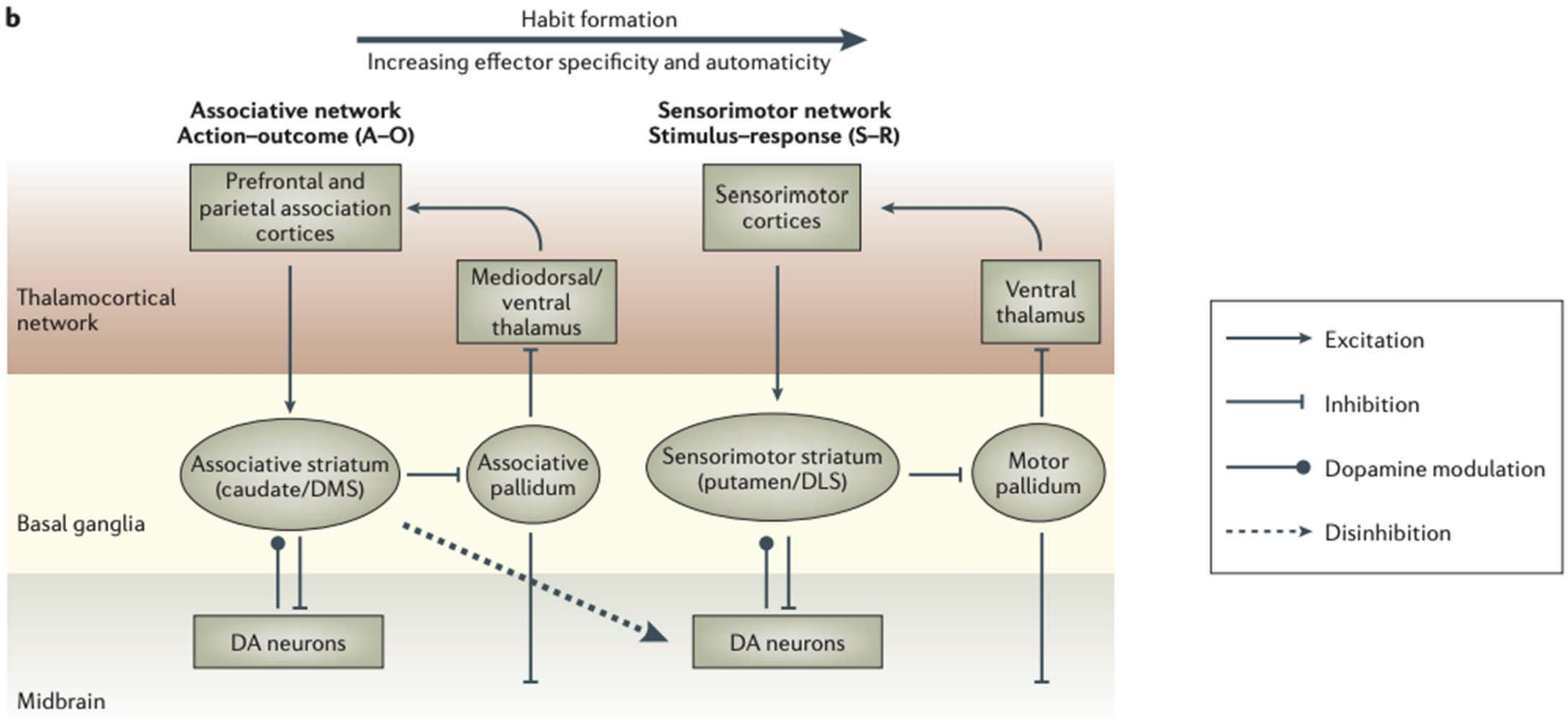
Habit formation - basal ganglia anatomy

Striatum (caudate and putamen)

- D1DR-expressing medium spiny neurons (MSNs) in striatum send **inhibitory** projections 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 in striatum 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 'D2pathway'.
- D2 system likely developed later in evolution, refining response selection mechanism

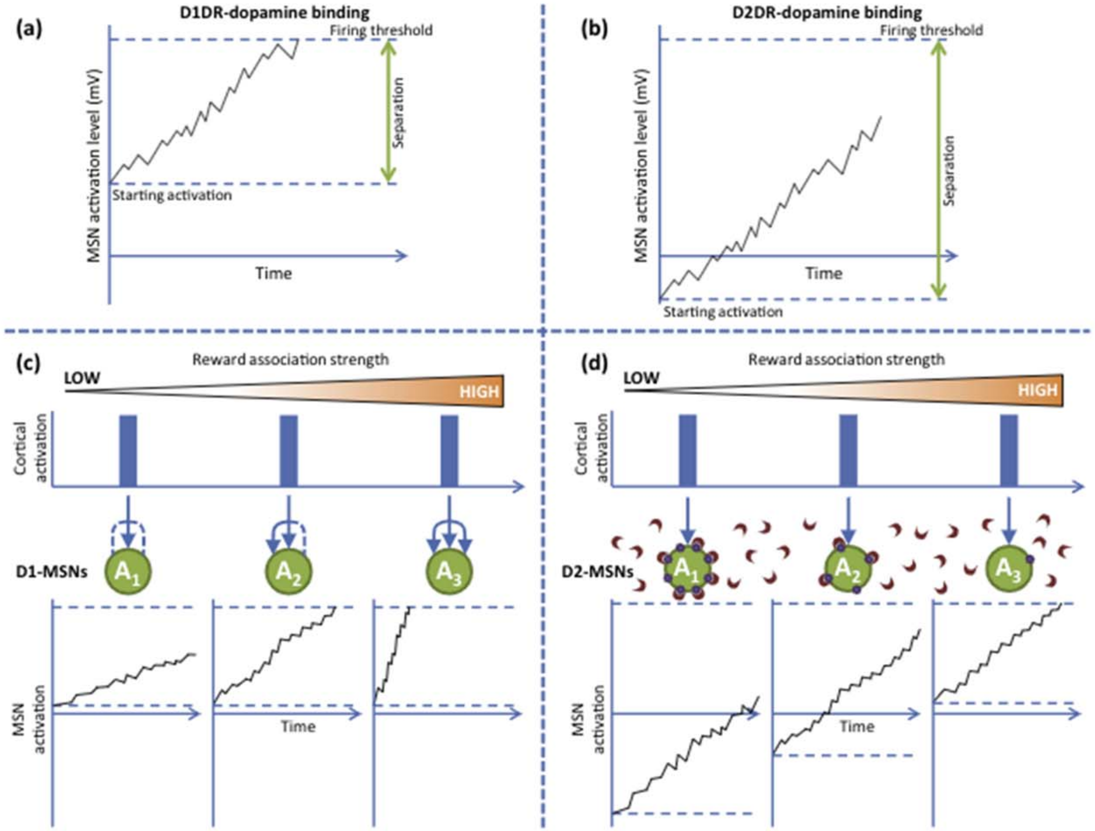


Habit formation



Habit formation - role of dopamine

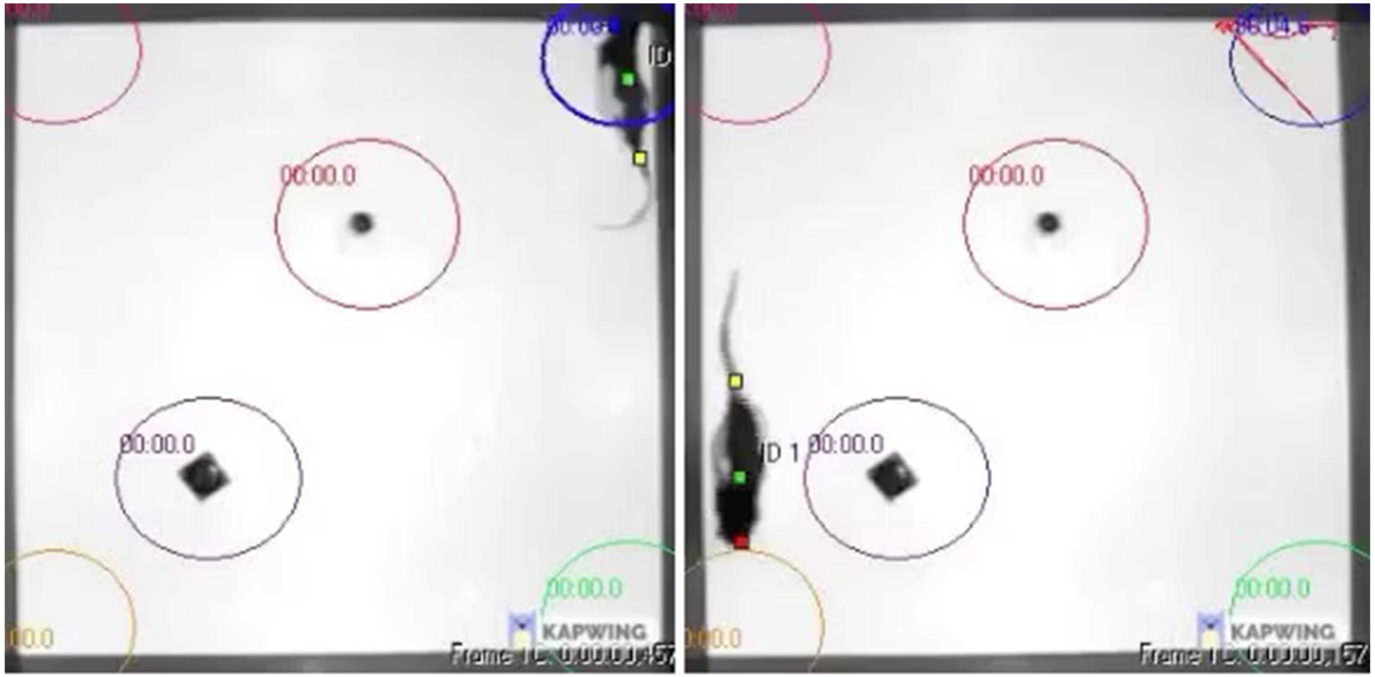
- D2 receptors are more sensitive dopamine therefore are always active - non-stop inhibition
- D1 receptors are less sensitive to dopamine, therefore higher dopamine level is needed to activate them
- Prepare and select model (PAS; Keeler et al., 2014)



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
- Inactivation of lesion of any part of the circuit can help 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



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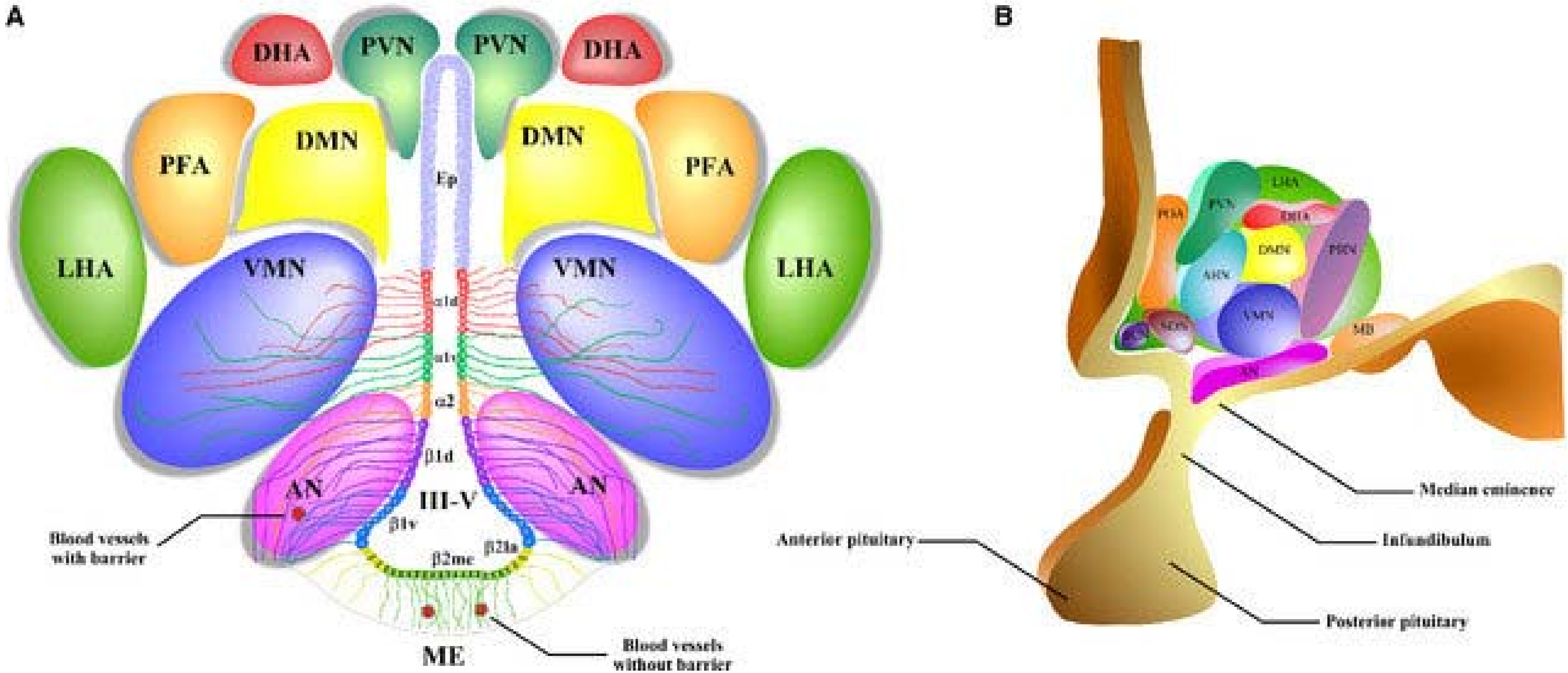
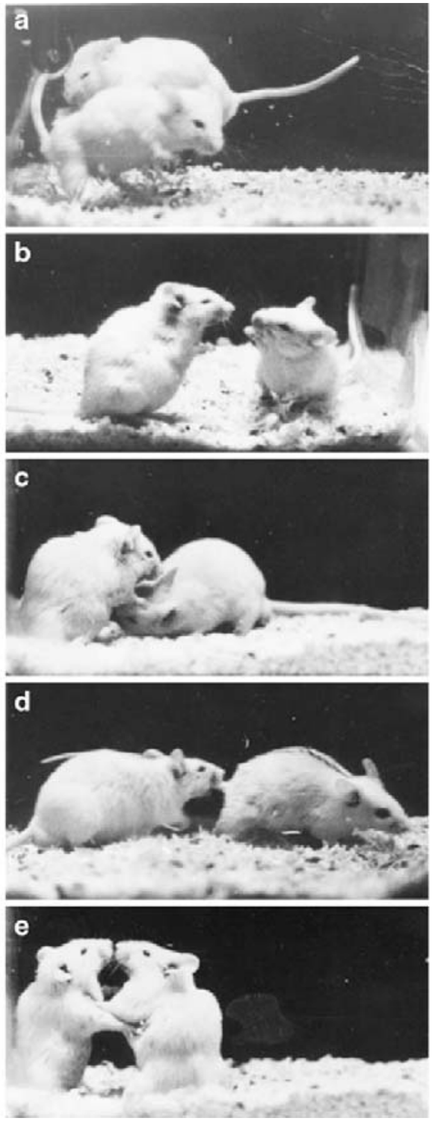
Innate behaviors: Hypothalamus



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
  - Perisitence 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))
- PMv processes sensory information
- Optogenetic activation of PMv triggers attack, optogenetic silencing PMv terminates attack
- Projects to HAA (includes 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 triggers 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

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

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
- Similarly to VMH, mPOA 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 of virgin males 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 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

Observing internal states in rodents

Vocalication

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

- Between rodents: ultrasound
- 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

- Induced by activation of dopamine D1, D2 and D3 receptors (all have to be activated concurrently)
- 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)
- 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
- Supressed by aversive stimuli
- 50kHz calls associated with release od dopamine from nucleus accumbens
- Most 50kHz calls when amphetamine is injected directly into nucleus accumbens

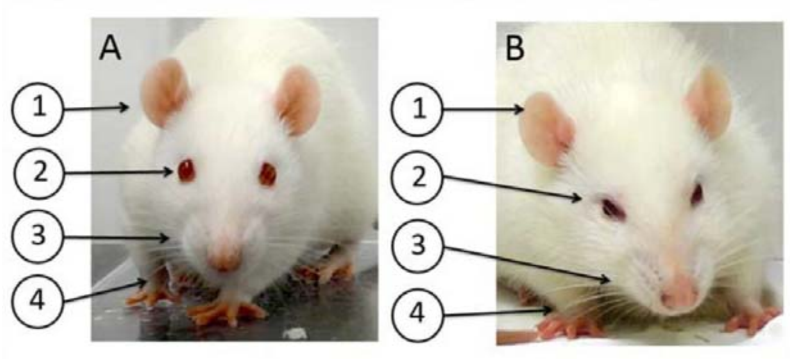
Vocalization - negative

- 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

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

- Neronal 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)
  - Positive arousal
- Negative calls:
  - Initiation: mesolimbic choligenic system 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
  - Negative arousal

Thank you!