Module 3: Social and Affective behaviors

Learning goal:

1) Why is it important to study natural behavior of animals to understand the brain?

2) What are the constraints of innate behaviors?

3) How are innate behaviors implemented, at behavioral, circuit and molecular/genetic levels?

Mon 29 Oct
Introduction to limbic system by John O’Keefe

Tues 30 Oct
Circadian Rhythm by Cristina Mazuski, **Neuromodulation and oxytocin** by Lennart Oettl

Fri 2 Nov
Innate social behaviors (incl. parental behavior) by Yoh Isogai

Mon 4 Nov
The amygdala by John O’Keefe

Tues 6 Nov
Defensive behavior by Tiago Branco

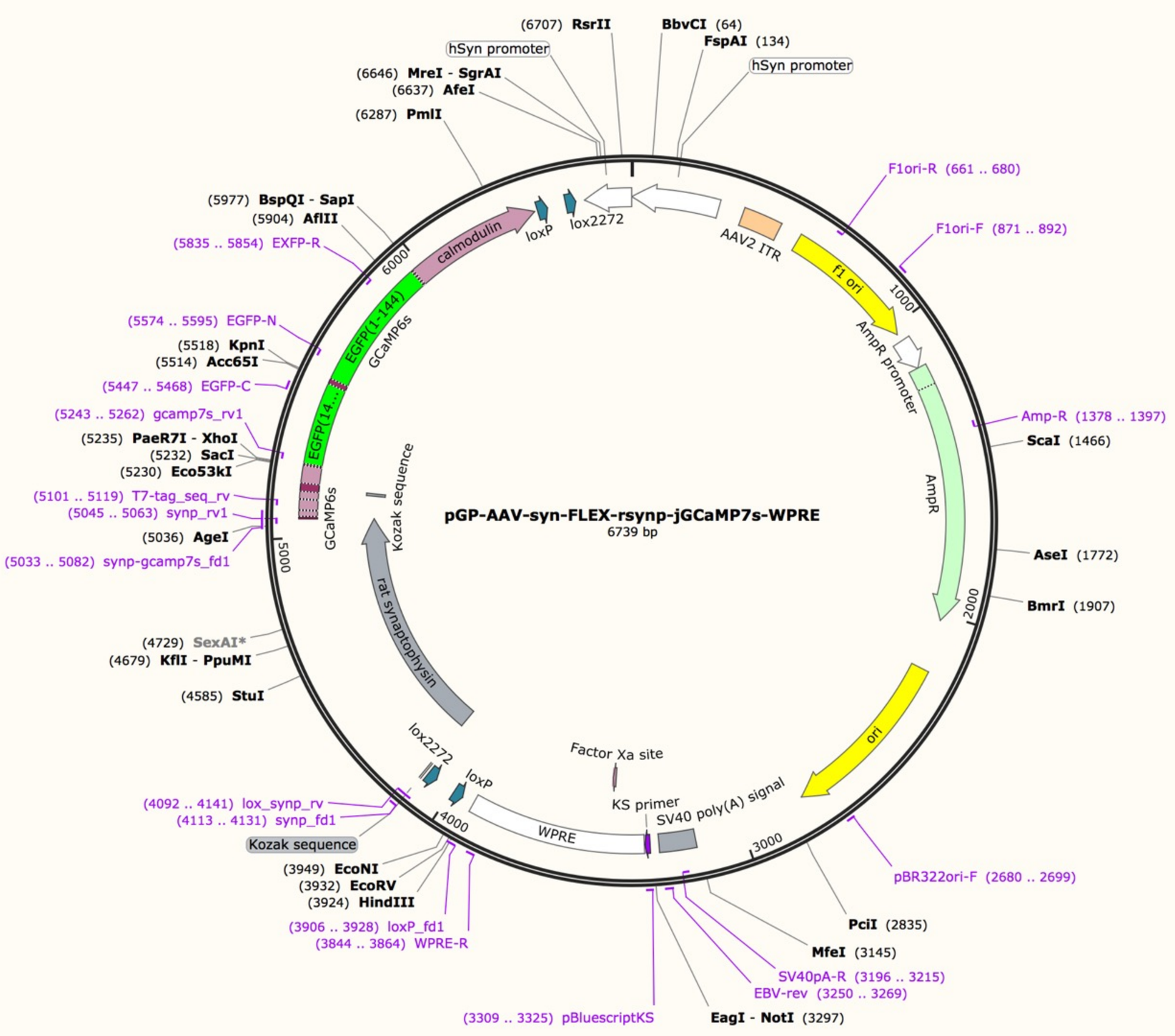
Fri 9 Nov
Human emotion by Quentin Huys

Experimental section

Instructors:
Mathew Edwards, Loukia Katsouri, Cristina Mazuski, Lennart Oettl, Daniel Regester

Goal:
1) To develop skills to analyze rodent behaviors
2) To acquire basic skills in molecular biology

1:30 pm today – Introduction to module 3 experimental section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6739 bp
(6707) RsrII
BbvCI (64)
FspAI (134)
hSyn promoter
hSyn promoter
(6646) MreI - SgrAI
(6637) AfeI
(6287) PmlI
F1ori-R (661 .. 680)
F1ori-F (871 .. 892)
(5977) BspQI - SapI
(5904) AflII
(5835 .. 5854) EXFP-R
calmodulin
loxP
lox2272
AAV2 ITR
f1 ori
AmpR promoter
AmpR
Amp-R (1378 .. 1397)
ScaI (1466)
AseI (1772)
BmrI (1907)
ori
pBR322ori-F (2680 .. 2699)
PciI (2835)
MfeI (3145)
SV40pA-R (3196 .. 3215)
EBV-rev (3250 .. 3269)
EagI - NotI (3297)
(3309 .. 3325) pBluescriptKS
SV40 poly(A) signal
KS primer
Factor Xa site
WPRE
loxP
lox2272
rat synaptophysin
Kozak sequence
GCaMP6s
EGFP(14...
EGFP(1-144)
GCaMP6s
(5574 .. 5595) EGFP-N
(5518) KpnI
(5514) Acc65I
(5447 .. 5468) EGFP-C
(5243 .. 5262) gcamp7s_rv1
(5235) PaeR7I - XhoI
(5232) SacI
(5230) Eco53kI
(5101 .. 5119) T7-tag_seq_rv
(5045 .. 5063) synp_rv1
(5036) AgeI
(5033 .. 5082) synp-gcamp7s_fd1
(4729) SexAI*
(4679) KflI - PpuMI
(4585) StuI
(4092 .. 4141) lox_synp_rv
(4113 .. 4131) synp_fd1
Kozak sequence
(3949) EcoNI
(3932) EcoRV
(3924) HindIII
(3906 .. 3928) loxP_fd1
(3844 .. 3864) WPRE-R
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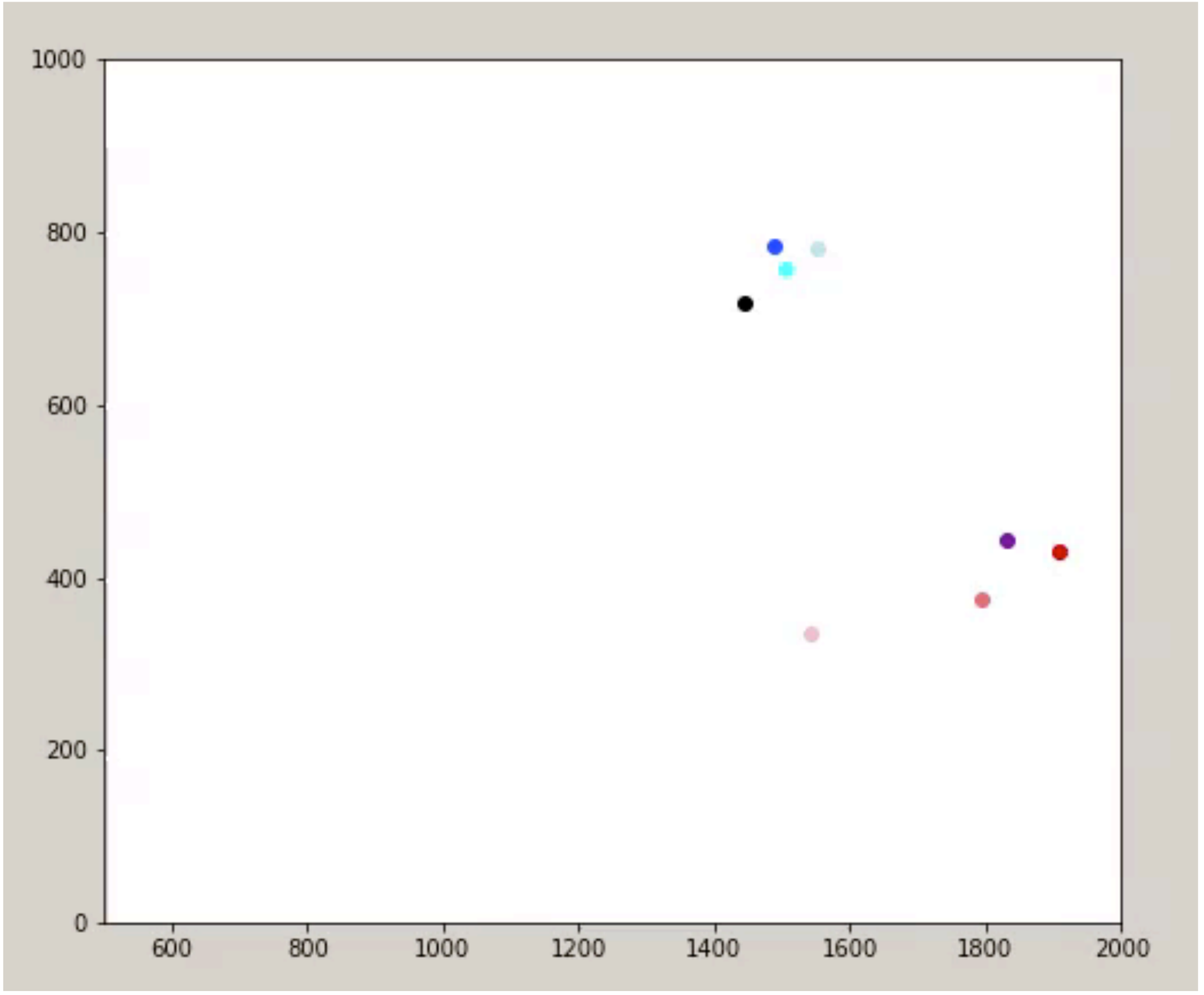
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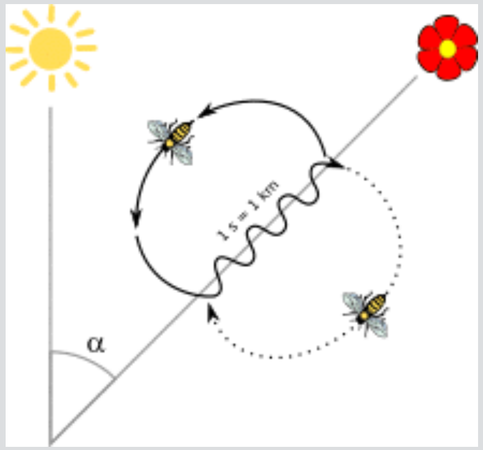
Ethology - study of animal behavior in natural conditions



Evolutionary constraints on behaviors

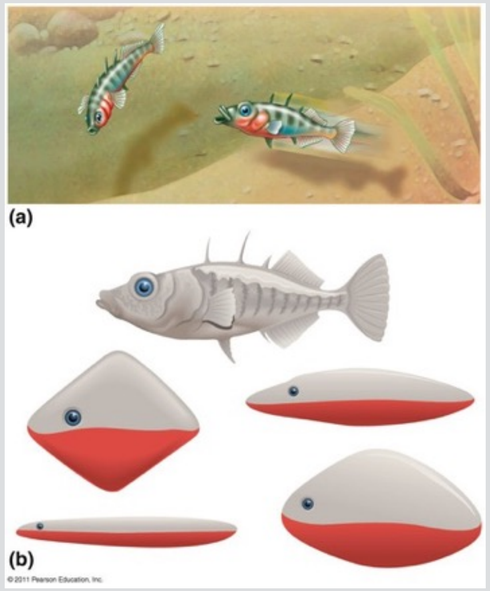
Survival behaviors:

Reproductive behaviors
Defensive behaviors
Parental care

Dedicated circuits

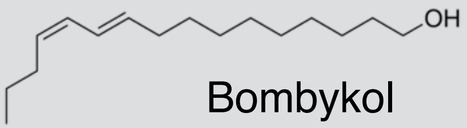
Sign stimuli

Physical features
(e.g., color)



(Tinbergen et al.)

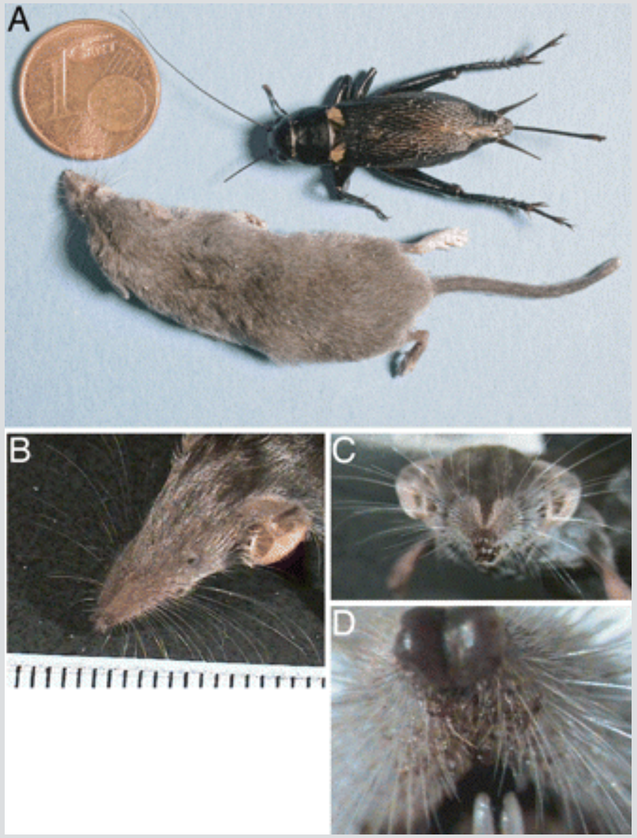
Chemosignals “**pheromones**”

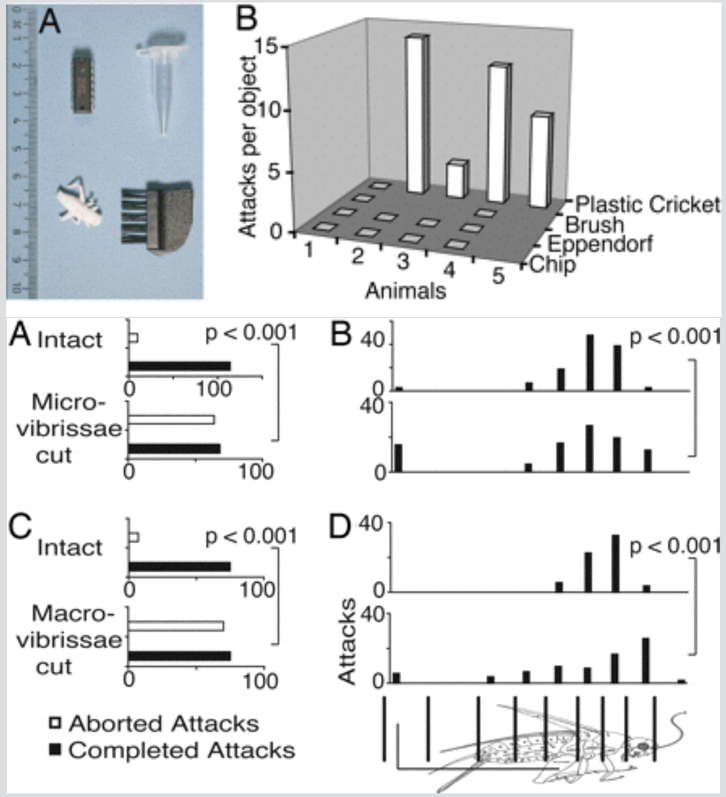


Bombykol
(Butenandt et al.)

More examples of sign stimuli

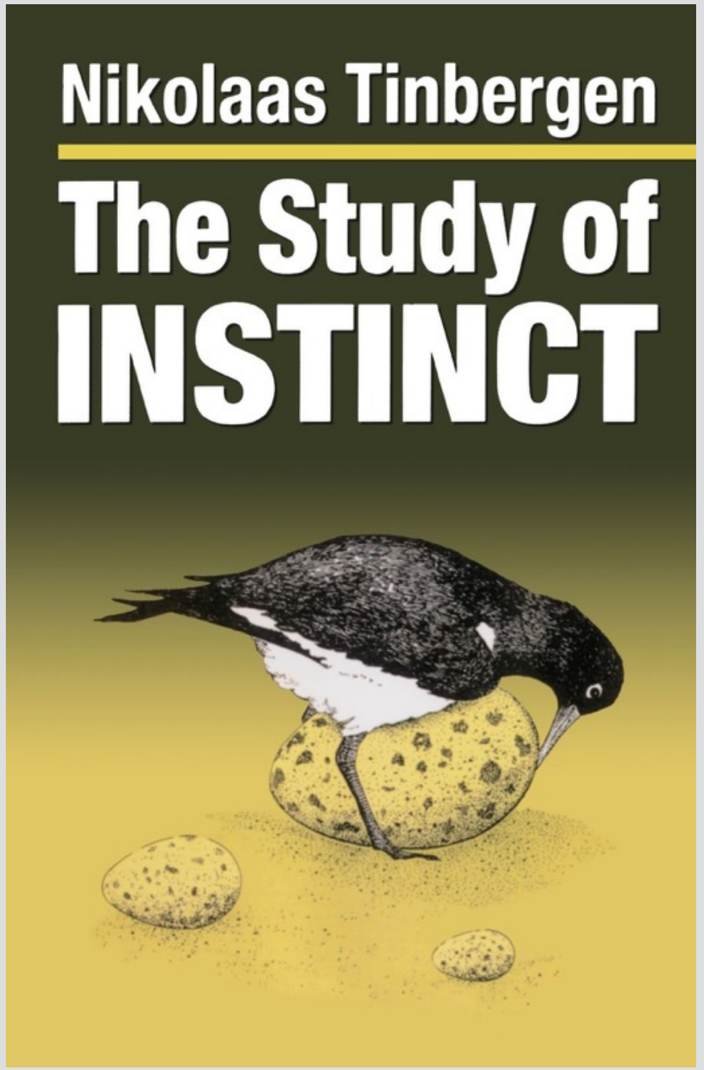
Tactile





(Anjum et al. *PNAS* 2006)

Dynamic range of sign stimuli

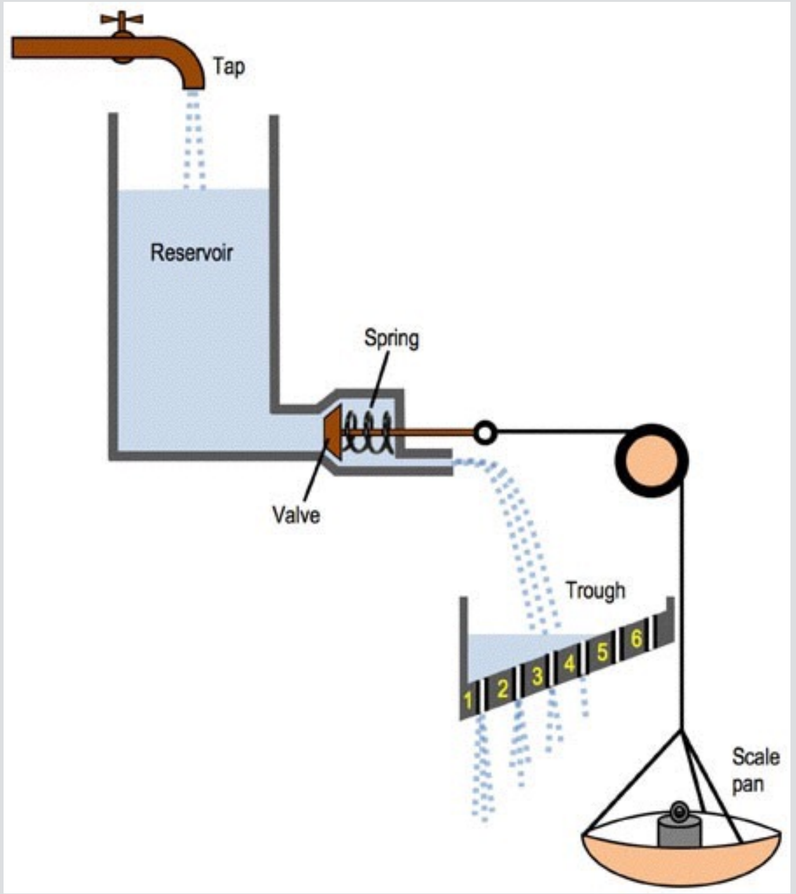


Theories of social behaviors

How do we explain:

- Innate drive: seemingly “analog”
- Effects by internal states, such as hormones
- Organization of behaviors

Lorenz’s model on motivation and drive



Neuronal circuit implementation?

Beach's model on interaction between sensory stimuli and internal states

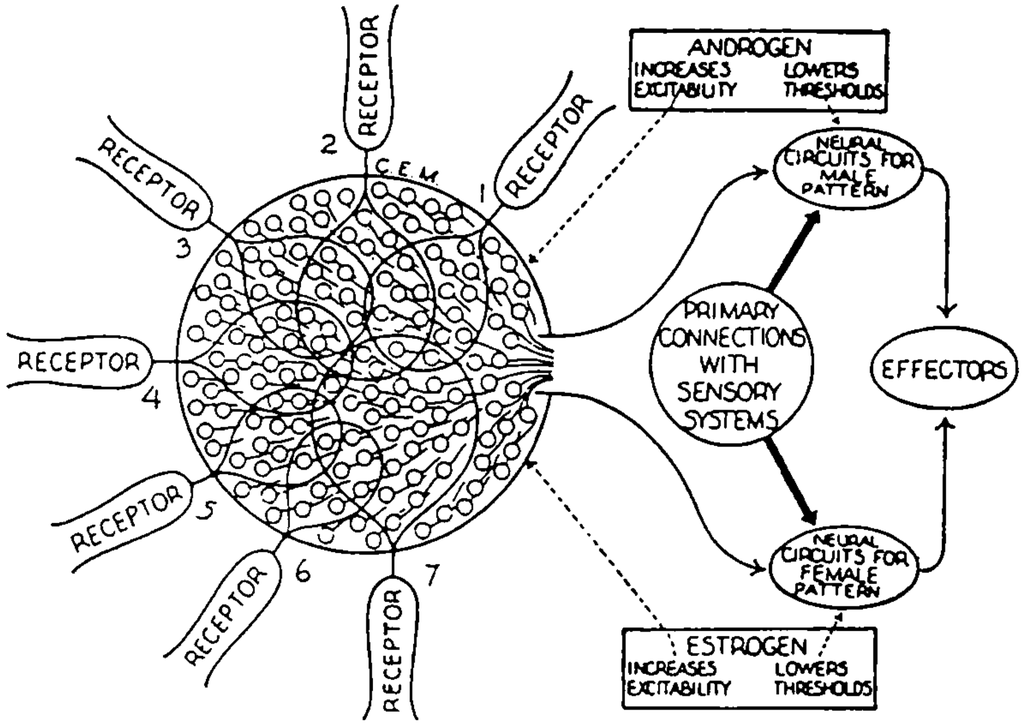


FIG. 2. Beach's 1942 depiction of the relationships between multisensory inputs, the "Central Excitatory Mechanism," and the motor circuits responsible for mating behavior in male and female mammals. Reprinted from (13), by permission.

????

Tinbergen's model on hierarchical organization of social behaviors

Motivation

Internal states

Sensory stimuli

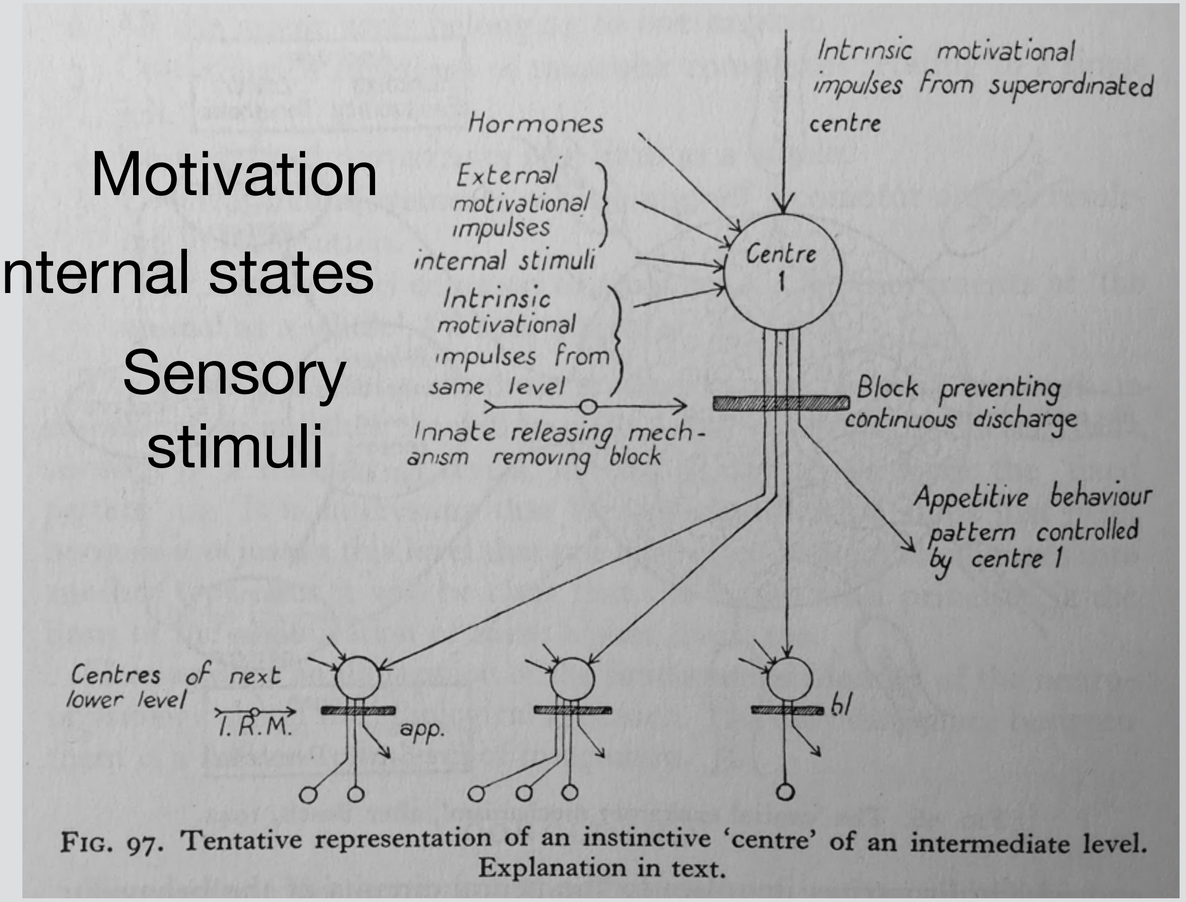
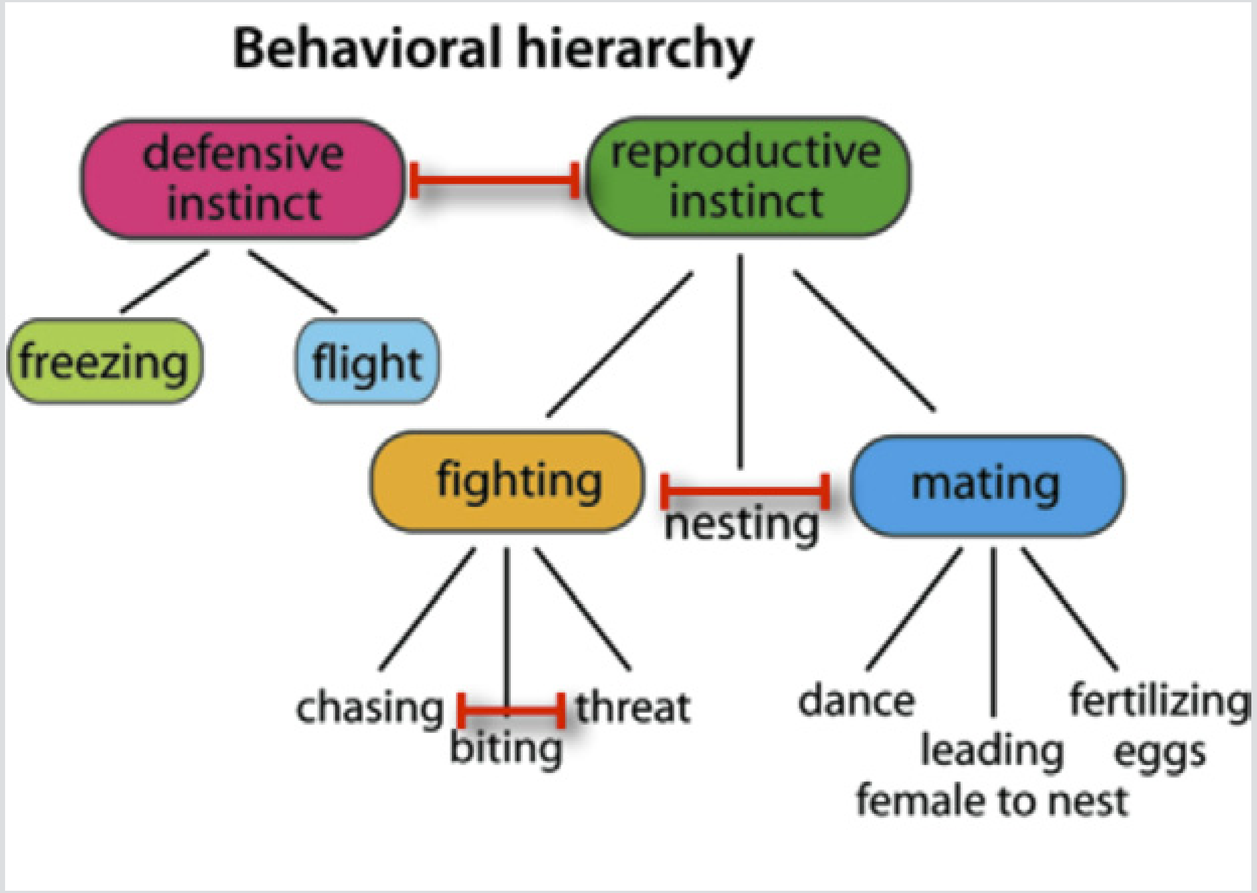
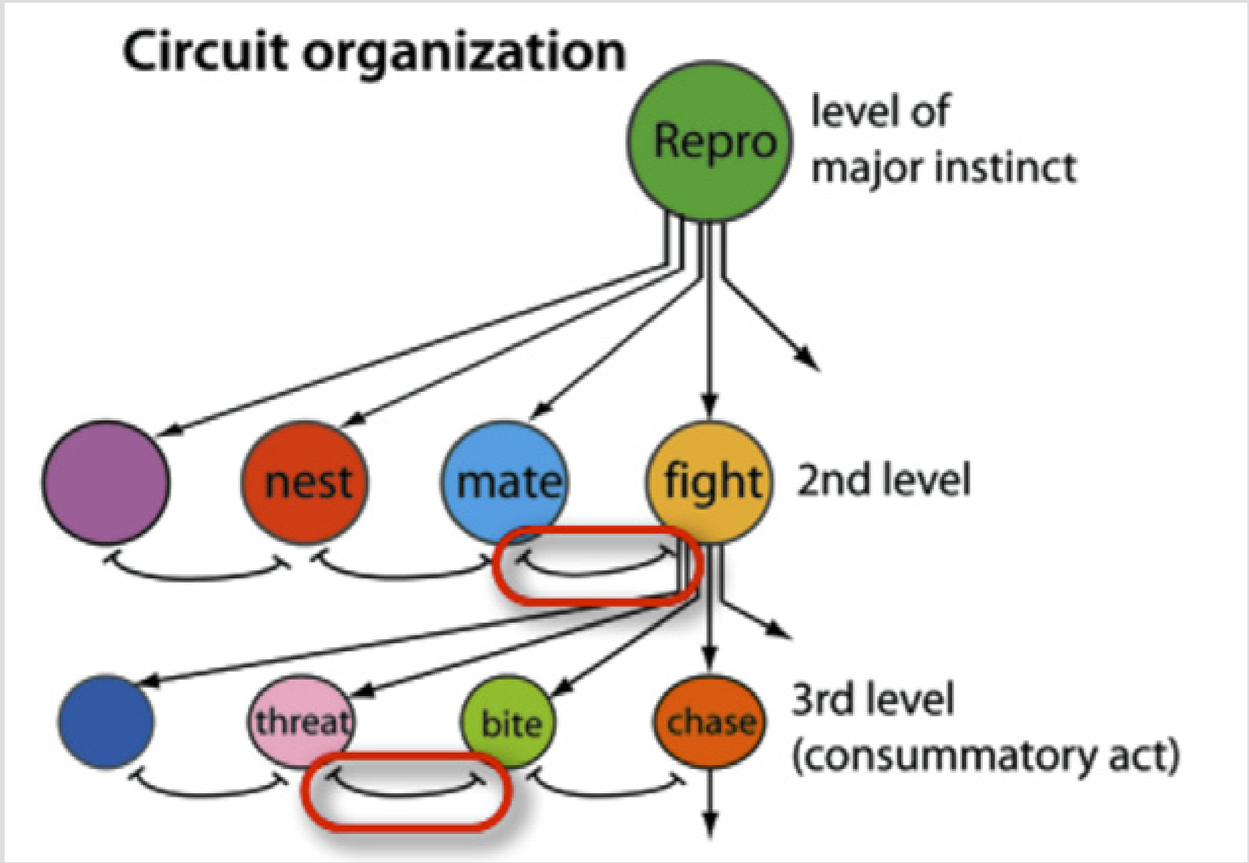


FIG. 97. Tentative representation of an instinctive 'centre' of an intermediate level. Explanation in text.

Organization of behaviors



A modern spin



(Anderson, *Biol Psychiatry* 2012)

The legacy of ethology

- Neuroethology: studies of highly specialized sensory/motor systems in animal kingdom:

- e.g., owl prey capture, stomatogastric ganglion in crabs, bat echolocation —fill in with your personal favorites—

- Sign stimuli: largely forgotten - being criticized as too simplistic?

Where do we go from here?

III

THE INTERNAL FACTORS RESPONSIBLE FOR THE 'SPONTANEITY' OF BEHAVIOUR

In every study of the releasing value of sensory stimuli one is faced by the phenomenon of a varying threshold. The very same stimulus that releases a maximal reaction at one time may have no effect at all or may elicit a weak response at another time. This variation of threshold could be due to either (1) a variation of the intensity of another external stimulus not controlled in the experiment, or (2) a variation of the intensity of internal factors, or (3) both. In this chapter we shall consider the internal factors. The effect of these internal factors determines the 'motivation' of an animal, the activation of its instincts.

The methods of collecting facts bearing on this problem are of different kinds. First there are indirect methods. These are of three types: (*a*) changes of intensity or frequency of a response are observed under constant conditions; (*b*) the minimum intensity of the stimulus necessary to release a response is determined at different times while the conditions are kept constant in every other possible respect; (*c*) the minimum intensity of a stimulus required to inhibit a reaction is measured and its variations in the course of time are observed (obstruction method). The work done in these fields is rather fragmentary; nevertheless the results are of considerable interest.

Secondly there is more direct evidence. This has been obtained by studying the effects of experimentally controlled changes within the animal.

While the indirect evidence has been collected by students of behaviour, the more direct method was used by neurophysiologists and endocrinologists. The contact between these two types of investigators has not been what it should be; as a consequence too few attempts have been made to arrive at a coherent picture, although several tentative steps have been taken.

INDIRECT EVIDENCE

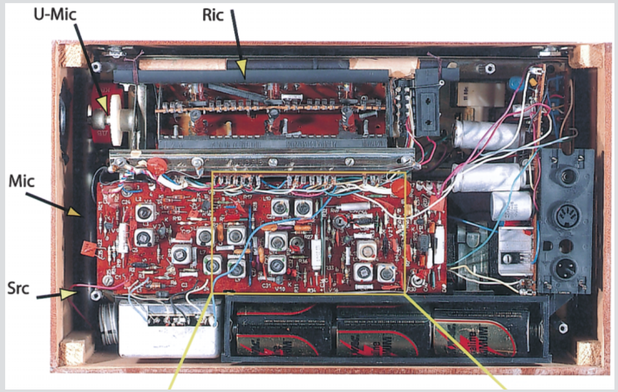
Variations of Intensity of Frequency of the Reaction under Constant Conditions

This phenomenon has been observed by many workers. However, very few careful and systematic studies have been made. Whitman (1919) summarized his extensive observations on the frequency of reproductive activities of pigeons in the course of the season in a

Tinbergen, The Study of Instinct, 1967

How do we “understand” the behaviors of a complex system?

A simple(-listic) case

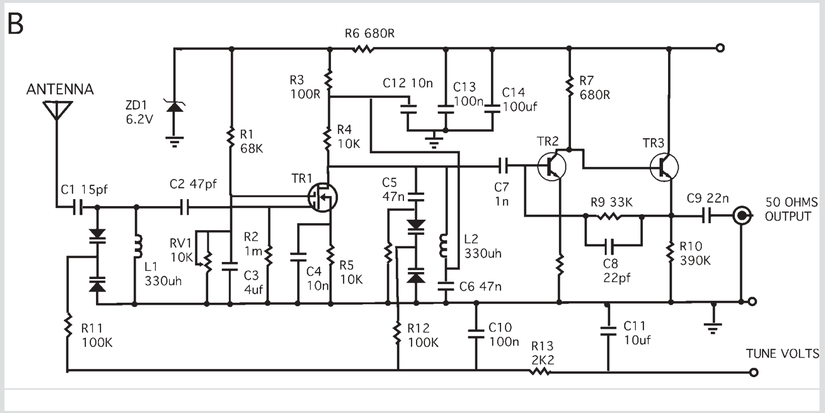


(Lazebnik, *Cancer Cell* 2002)

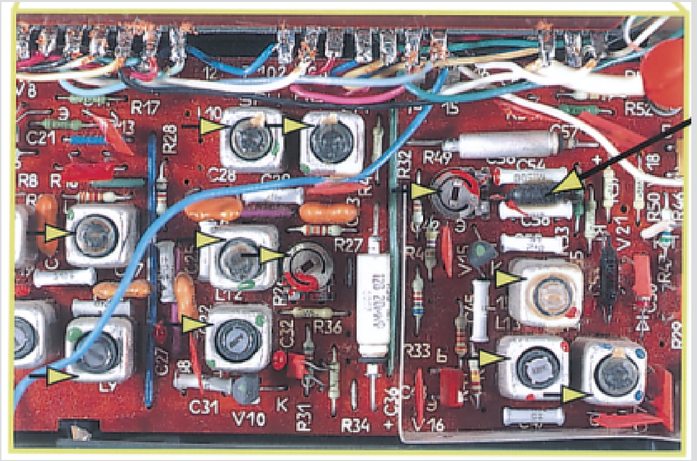
How would you try to understand how a radio works?

Analysis of a complex machinery requires several levels of inquiries

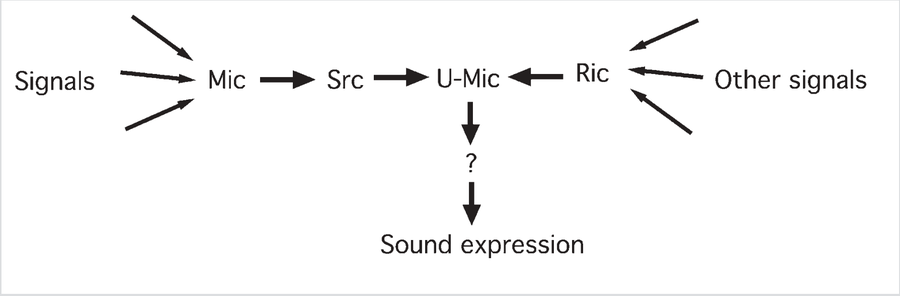
Structure/Wiring diagram

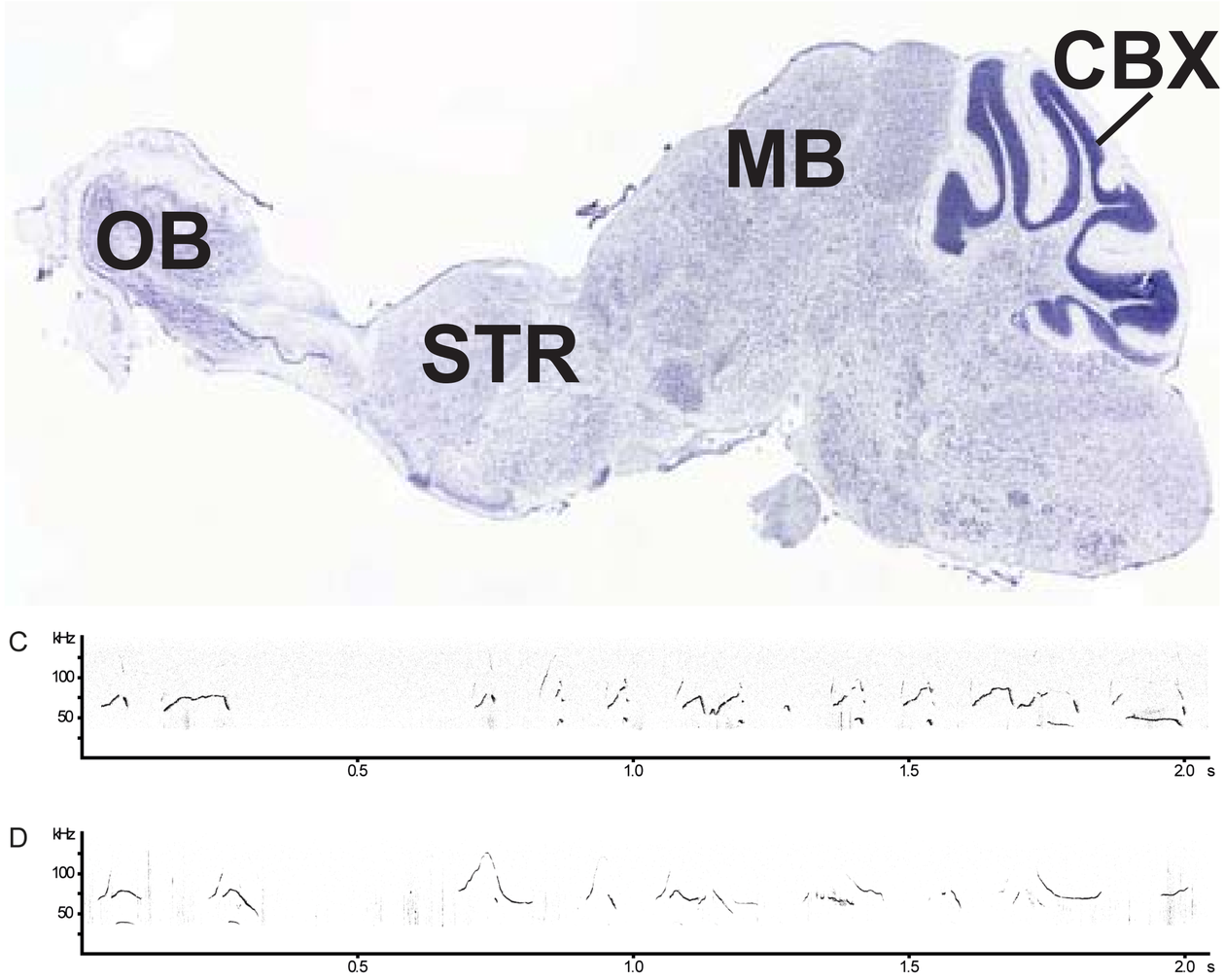


Functional studies



Model





(Hammerschmidt et al. *Sci Rep* 2015)

Genes and Social Behavior

2 Nov 2018

Yoh Isogai

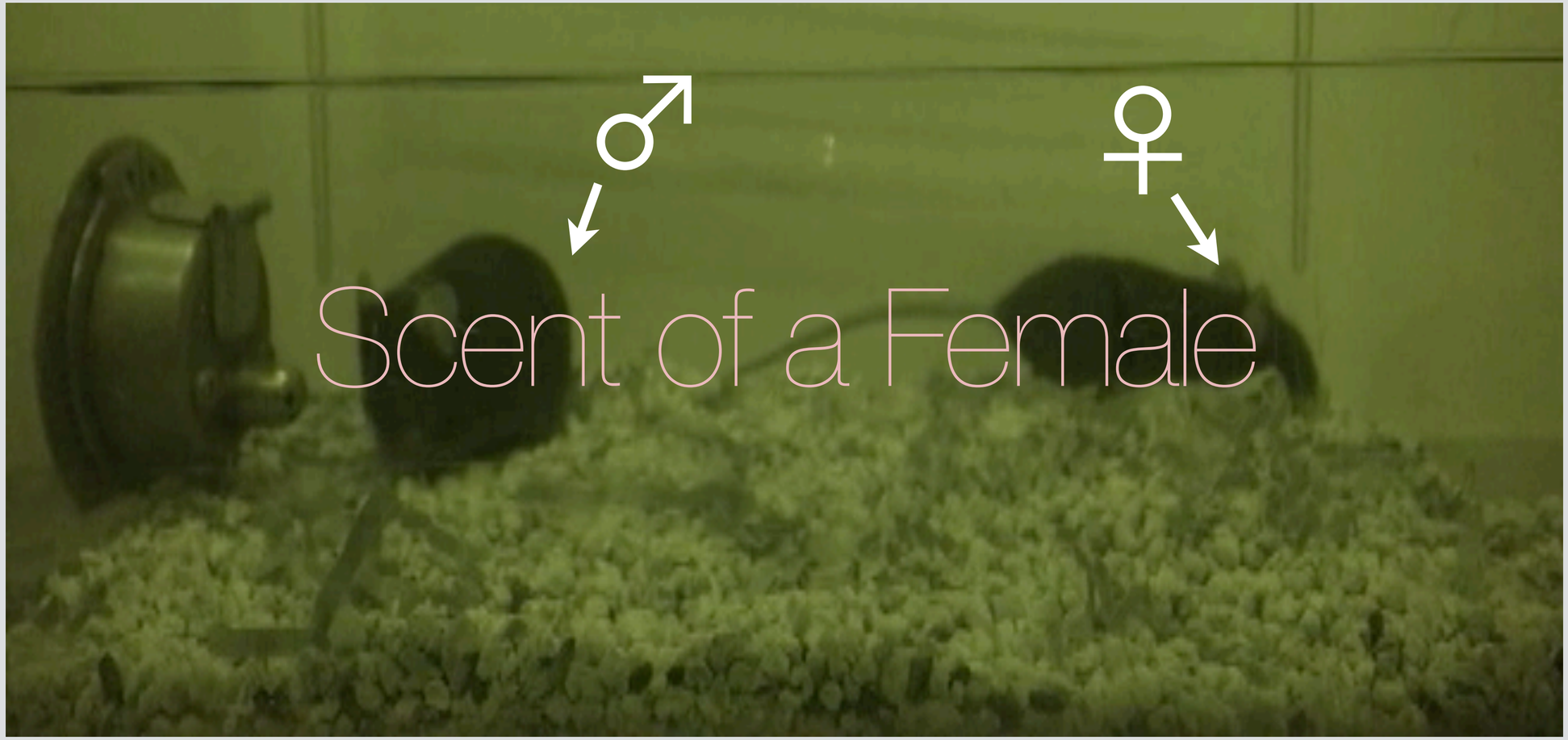
Today’s agenda

- What are the components of neural circuits underlying social behavior?

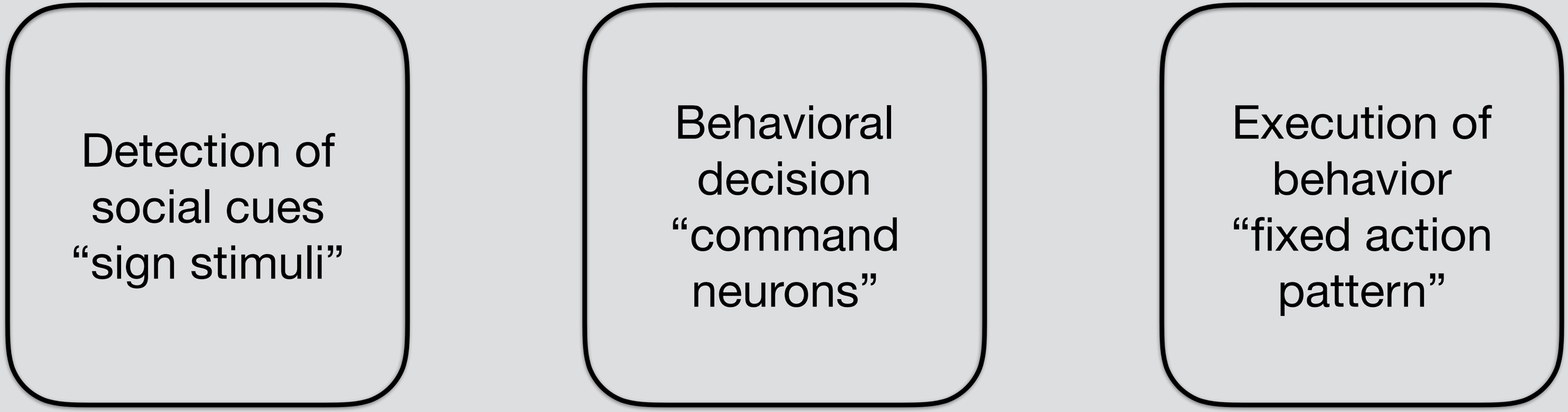
- Parts list of social behavior circuits

- Discussion - Top down vs bottom up approaches

How do odors trigger innate behaviors?



Phases of social behaviors



Keep in mind: each social behavior consists of multiple components

Drosophila courtship behavior

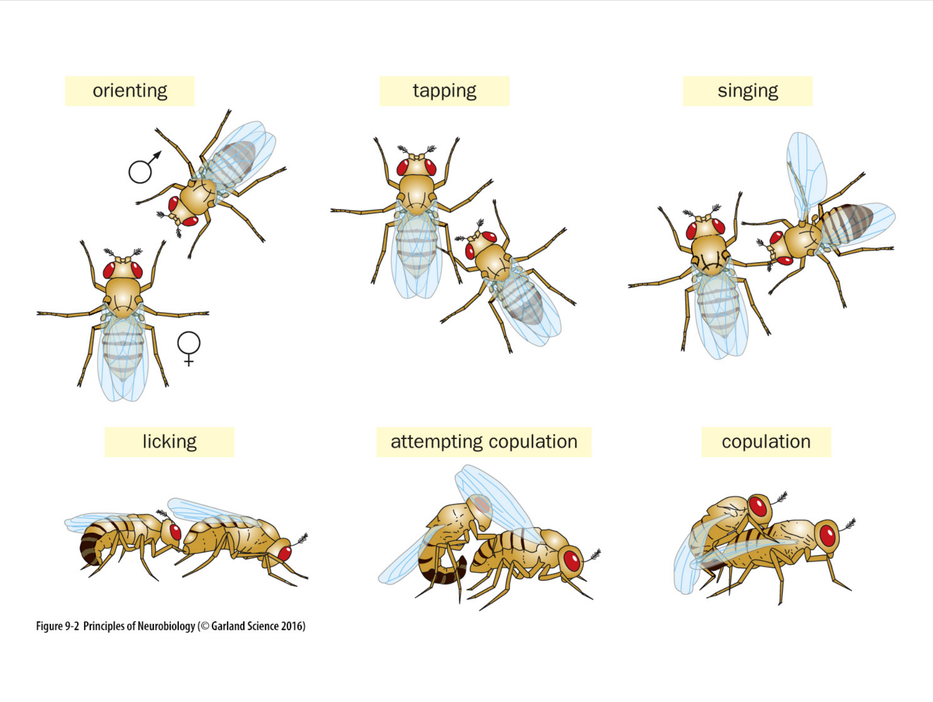
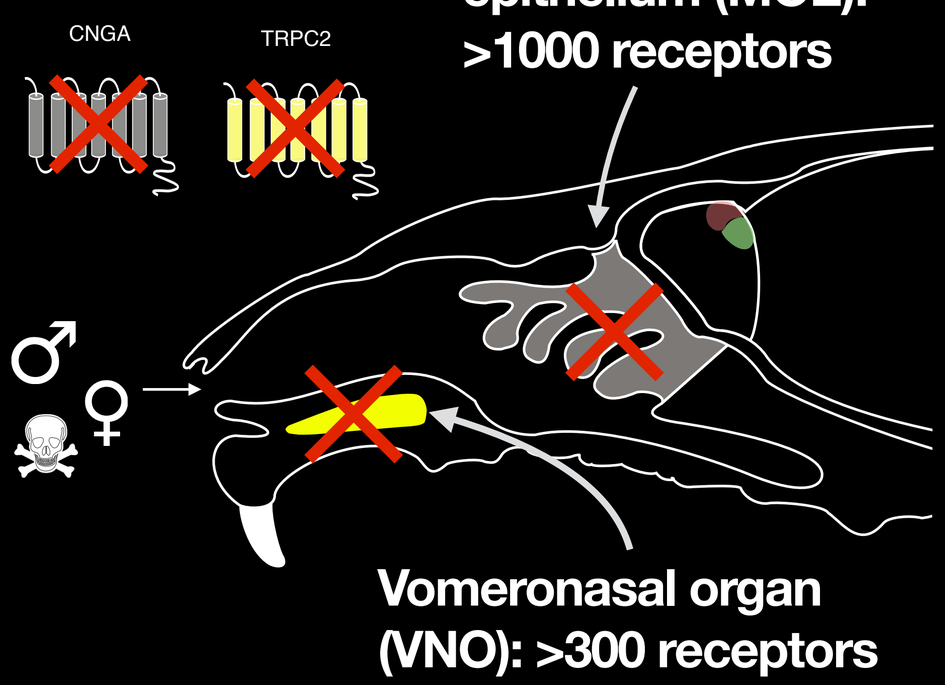


Figure 9-2 Principles of Neurobiology (© Garland Science 2016)

Parental behavior

What are social cues?

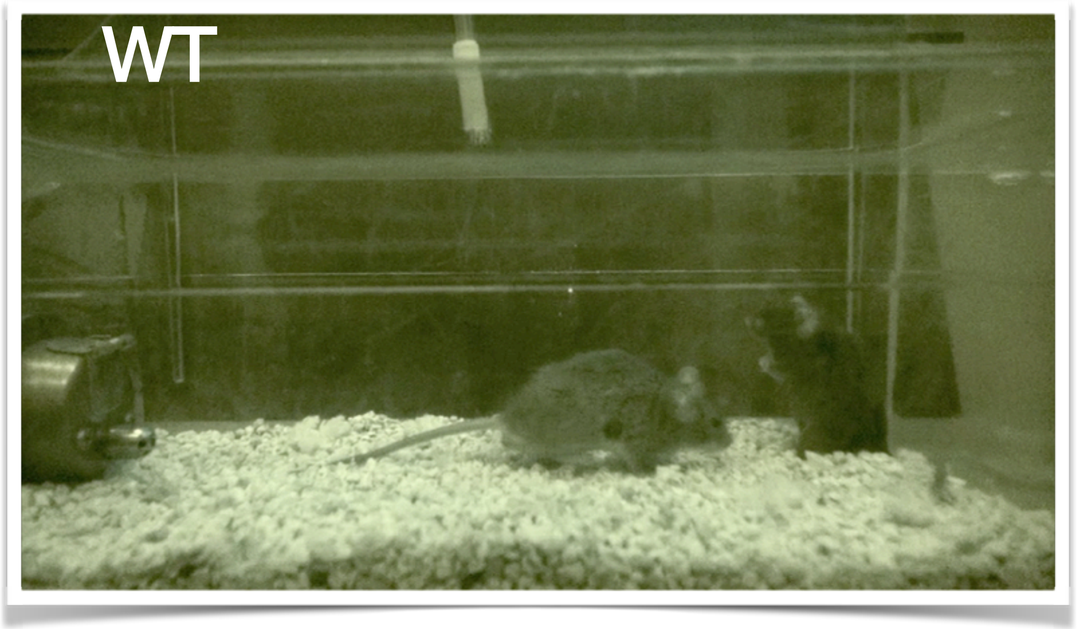
Olfactory information drives social behaviors

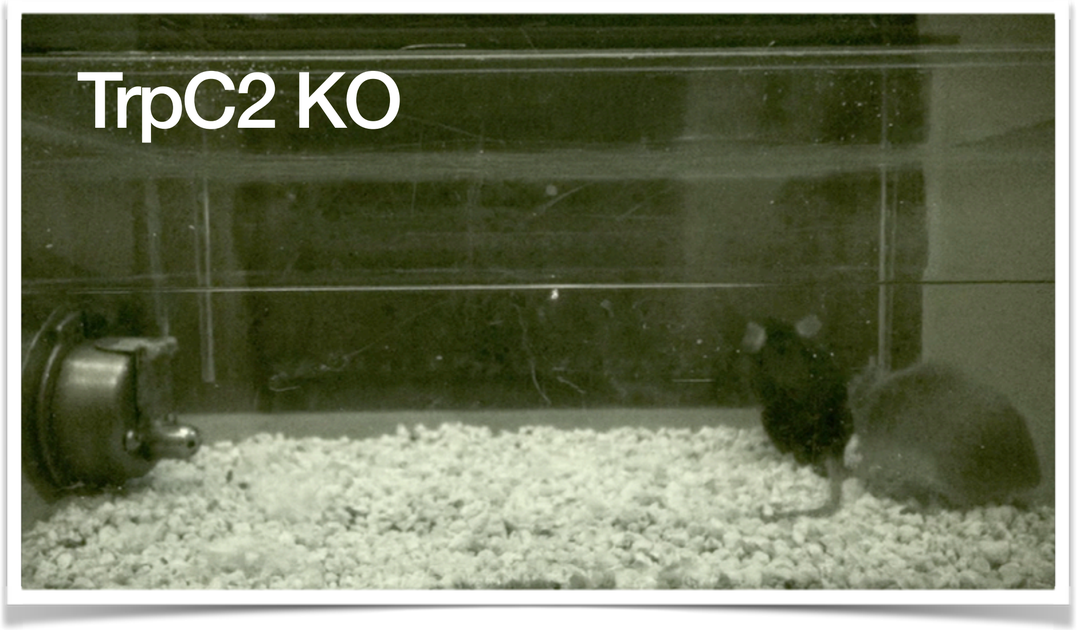


Loss of sex discrimination, aggression, innate fear

(Stowers et al. *Science* 2002, Leypold et al. *PNAS* 2002, Papes et al. *Cell* 2010)

The vomeronasal organ is critical for sex discrimination





Wrong sensory information results in inappropriate behavior!

(Stowers et al. 2002; Leypold et al. 2002)

Social cues are multisensory

STUDIES OF MATERNAL RETRIEVING IN RATS. III. SENSORY CUES INVOLVED IN THE LACTATING FEMALE'S RESPONSE TO HER YOUNG [1])

by

FRANK A. BEACH and JULIAN JAYNES
(Department of Psychology, Yale University)

(With 3 Figs)
(Rec. 16-XI-1955)

INTRODUCTION

A central problem in the analysis of complex patterns of behavior is identification of the evoking stimuli. This is especially important in connection with those "species-specific" types of behavior which are usually termed "instinctive". As LASHLEY has expressed it:

TABLE I

Stimulus Object	*Sum of Such Objects Presented in All Tests Combined*	*Number of Females Tested*	*Number of Tests*	*Per Cent of Objects Retrieved*
Normal pup	177	18	33	98
Freshly killed up	54	17	54	95
Live pup covered with vasoline	25	9	13	64
Freshly killed pup half covered with collodion	34	9	17	53
Freshly killed pup completely covered with collodion	41	14	22	17
Dead pup preserved in alcohol	22	8	10	17
Piece of raw beef heart	64	18	31	14

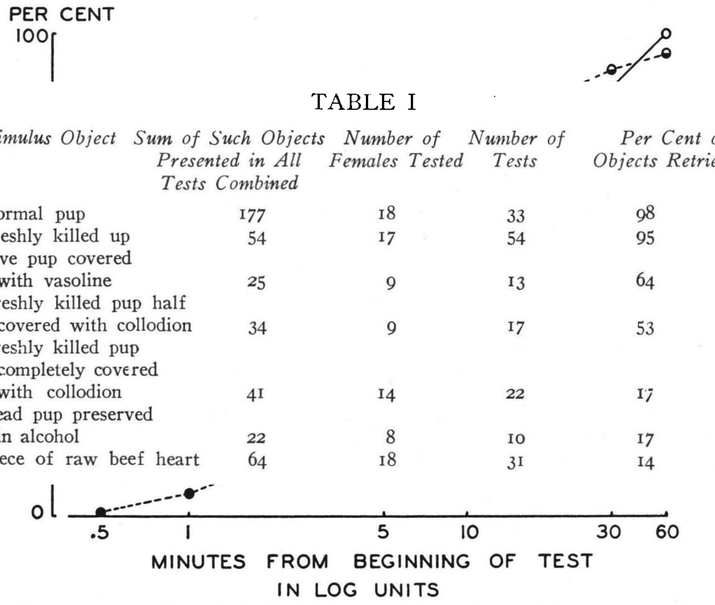


Fig. 2. Per cent of normal, freshly-killed, and refrigerated pups retrieved at the end of successive time intervals.

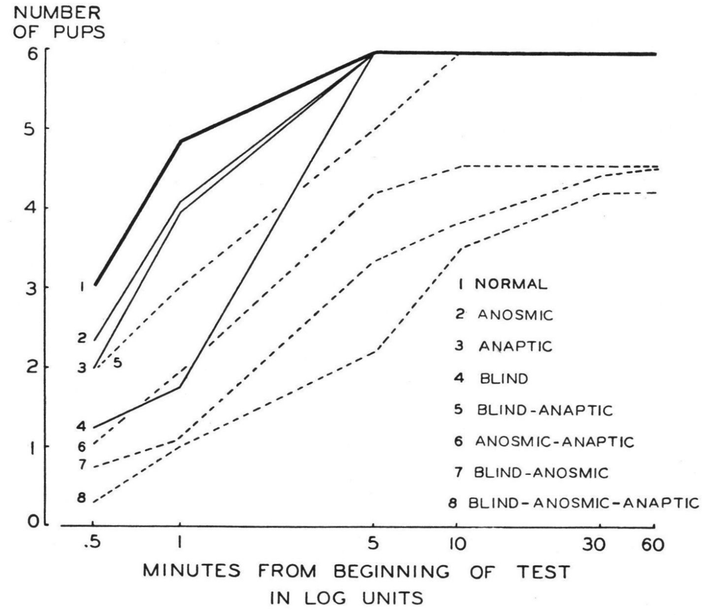


Fig. 3. Composite graph showing number of normal pups retrieved by females suffering various types of peripheral desensitization.

What is a pup?

- Smell
- Vocalization
- Shape
- Texture
- Color
- Temperature
- Motion

How do we delineate neural circuits underlying social behavior?

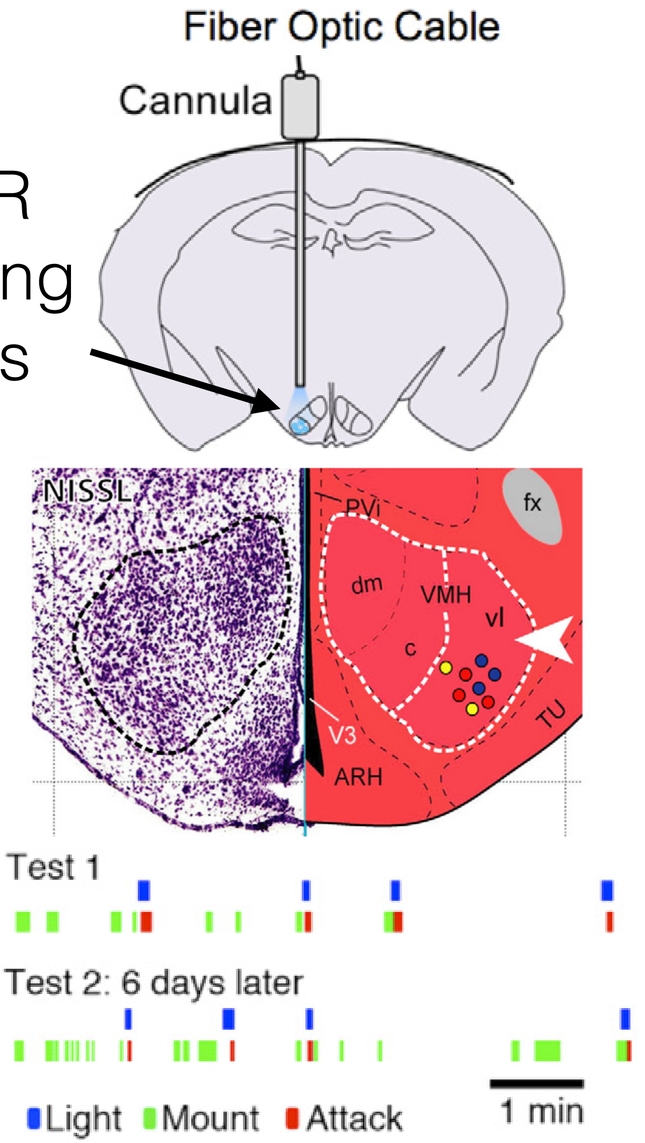
social cues 1 -> ? →

social cues 2 -> ? →

social cues 3 -> ? →

Control of aggression and mating by VMH neurons

ER/PGR expressing neurons



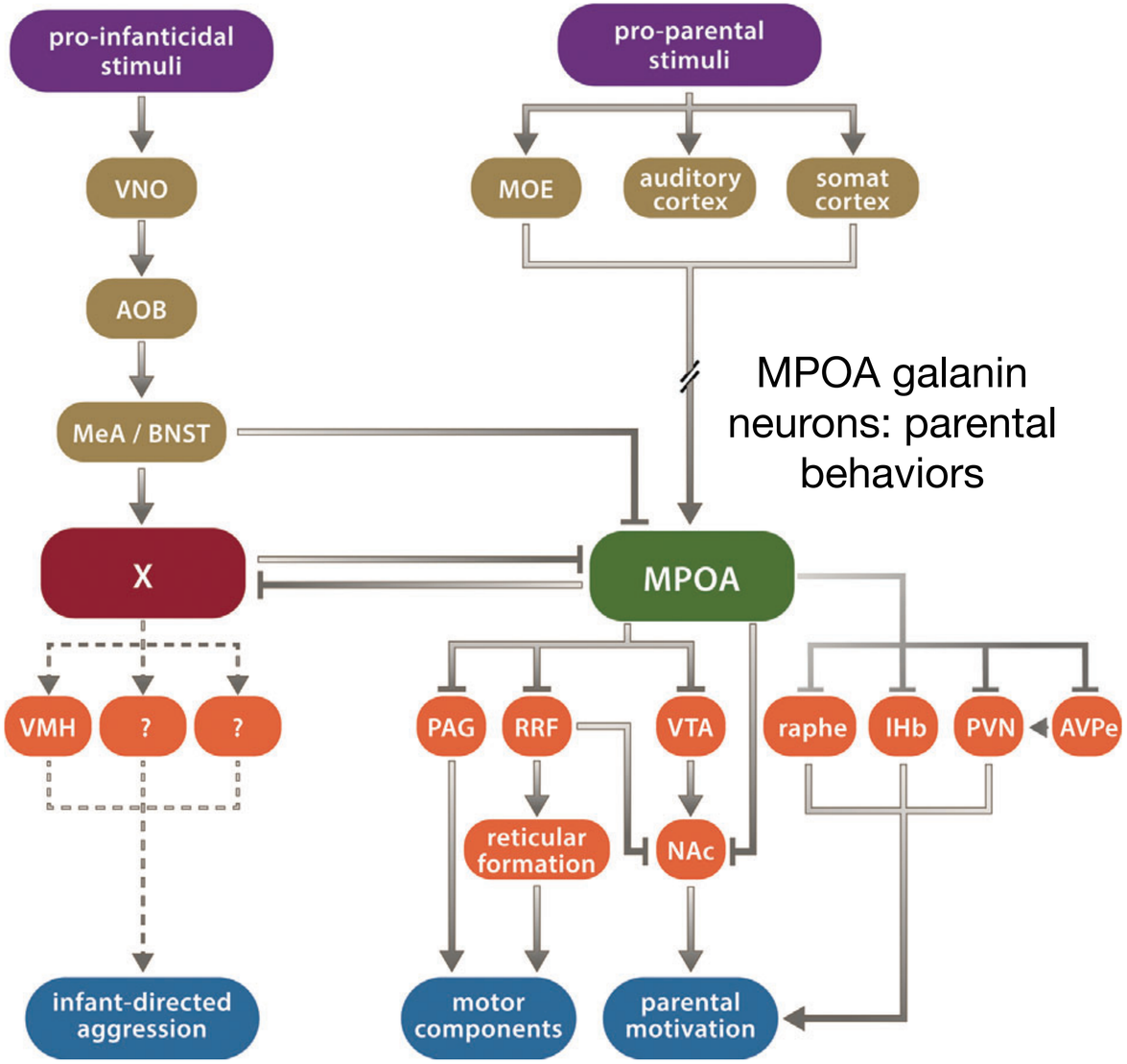
(Lin et al. *Nature* 2011)

Parental behavior

Regulation of parental behaviors

pan-MPOA^{Gal} fiber photometry recording (mother)

optogenetic activation of MPOA^{Gal}->VTA projections (virgin female)



(Kohl et al. *Nature* 2018, Kohl et al. *Bioessays* 2017)

Hypothalamic nuclei for execution of survival behaviors can be genetically defined

Hunger	Arc- Agrp
Thirst	MnPO- Adcyap1, Agtr1a, Nxph4
Mating/ Aggression	VMH- Estrogen receptor, progesterone receptor
Parental behavior	MPOA- Galanin

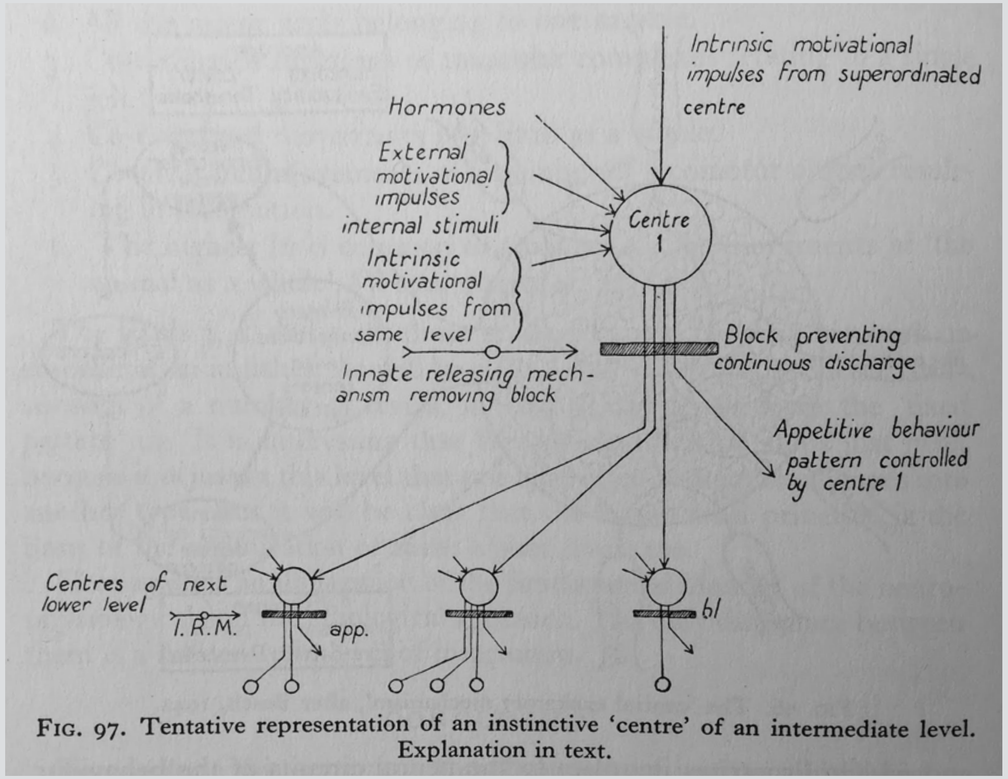
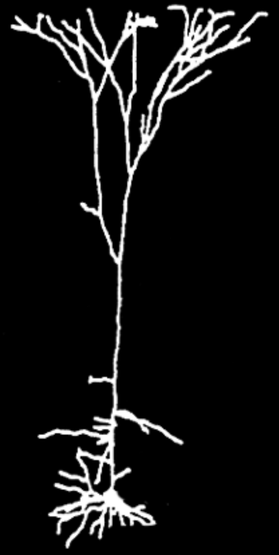
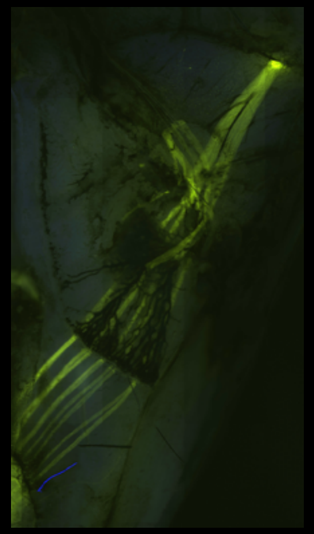


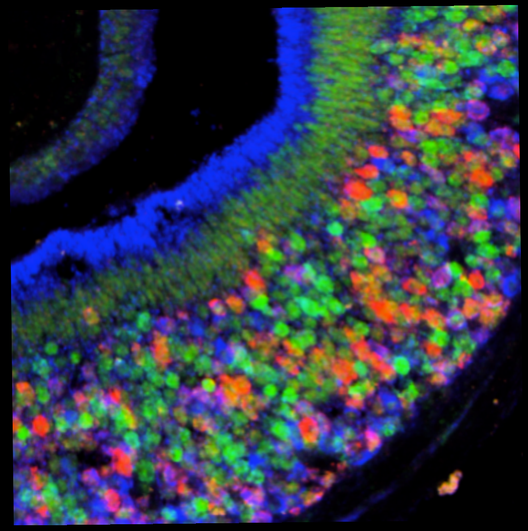
FIG. 97. Tentative representation of an instinctive 'centre' of an intermediate level. Explanation in text.

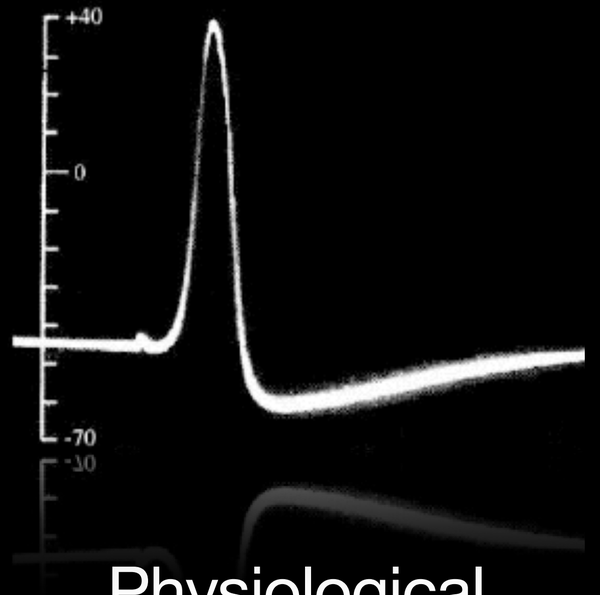
Tip of the iceberg - we have absolutely no idea what parts exists in the circuit!

What determines a “cell type”?









Anatomical

“Cell adhesion/ guidance molecules”

Molecular

“Genes”

Physiological

“Ion channels”

Behavioral

?

Top down vs bottom up

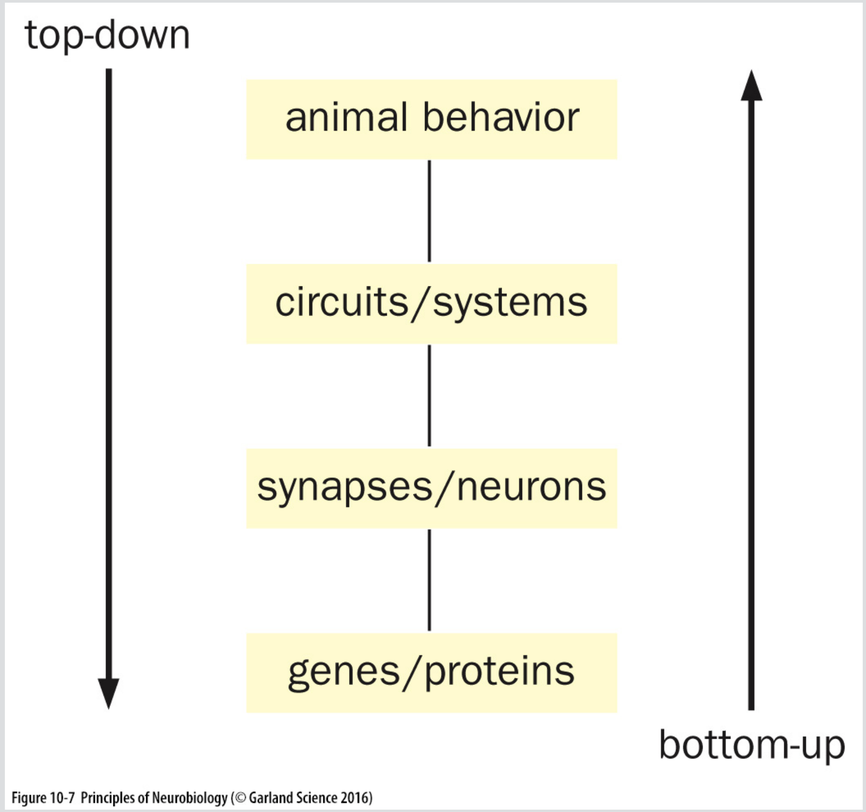
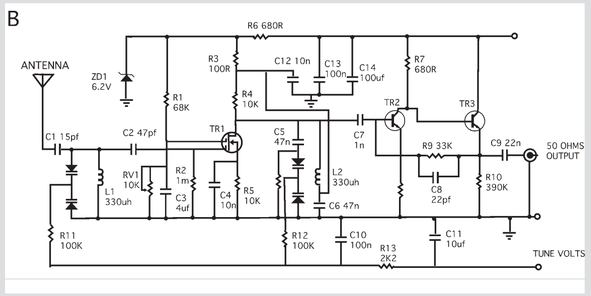
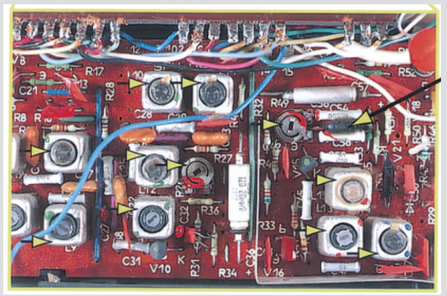


Figure 10-7 Principles of Neurobiology (© Garland Science 2016)

- When is reductionism useful?

- When is reductionism not effective?





Existential crisis of molecular neuroscience?

Did bottom up approaches yield meaningful understanding of the brain?

Highly recommended reading:

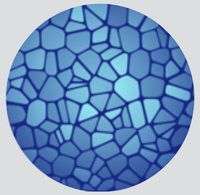
- Sudhof, *Neuron* 2017
- Sanes and Lichtman, *Nature Neurosci* 1999

Molecular taxonomy: classification of cells in the brain

Is the hype justified?

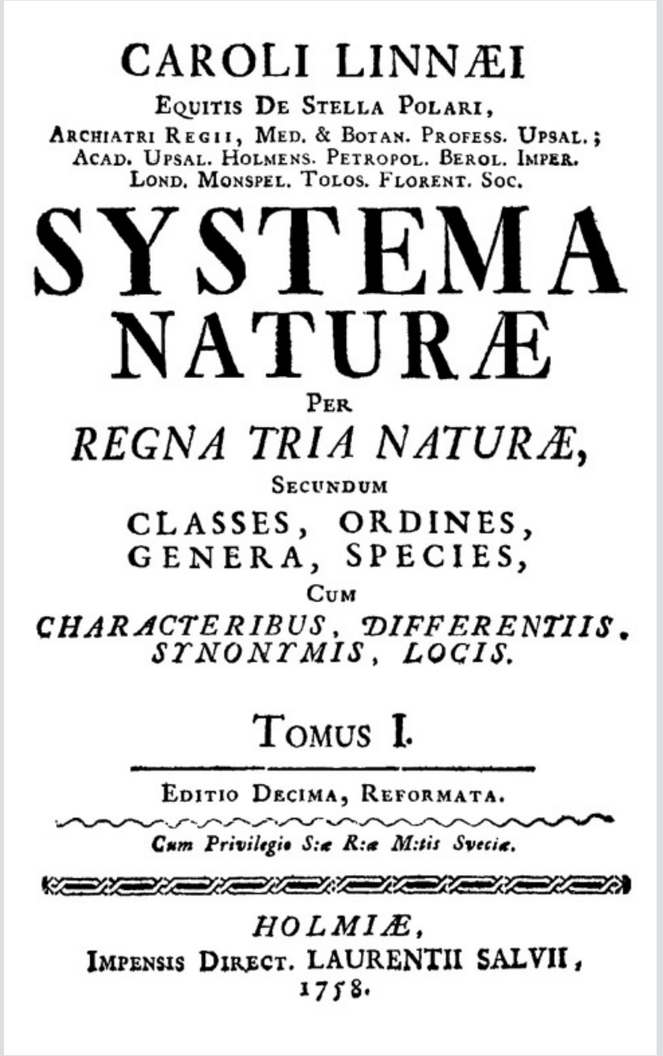
Why do we need a parts list?

How is a parts list used to help us understand the circuits underlying social behavior?



HUMAN CELL ATLAS

www.humancellatlas.org



CAROLI LINNÆI

EQUITIS DE STELLA POLARI,

ARCHIATRI REGII, MED. & BOTAN. PROFESS. UPSAL.;
ACAD. UPSAL. HOLMENS. PETROPOL. BEROL. IMPER.
LOND. MONSPEL. TOLOS. FLORENT. SOC.

SYSTEMA NATURÆ

PER

REGNA TRIA NATURÆ,

SECUNDUM

CLASSES, ORDINES,
GENERA, SPECIES,

CUM

CHARACTERIBUS, DIFFERENTIIS,
SYNONYMIS, LOCIS.

TOMUS I.

EDITIO DECIMA, REFORMATA.

Cum Privilegio S:æ R:æ M:tis Sveciæ.

HOLMIÆ,

IMPENSIS DIRECT. LAURENTII SALVII,
1758.

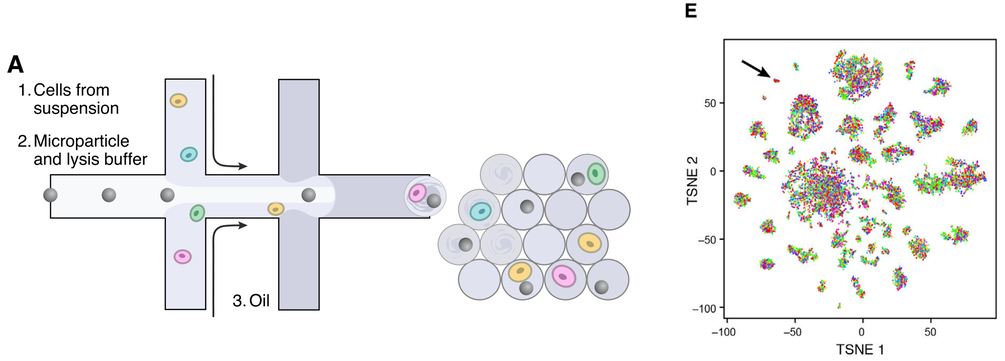
Let’s not forget that parts list is only the beginning!

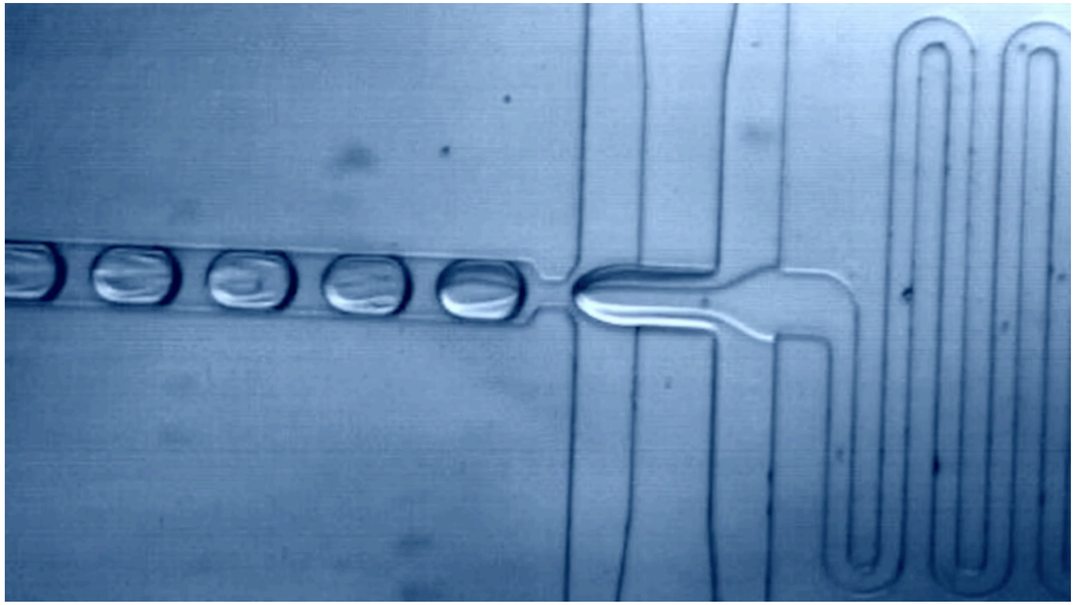
I was once told by one of the leaders in the field that the neurotransmitter that mediated a synaptic connection was irrelevant, and the only thing that mattered was the sign of the synapse, excitatory or inhibitory. Although today’s anatomists must know that neuromodulatory neurons can release their co-transmitters at a distance from their targets, the underlying assumption of today’s electron microscope connectome projects is that the conventional close-apposition synapses provide most, if not all, of the information needed to characterize the circuit, the same assumption that was made 35 years ago by the small-circuit physiologists.

(Marder, *Neuron* 2012)

Cell type classification by gene expression

Single cell RNA (scRNA)-seq





(Macosko et al. Cell 2015)

Multiplexed RNA fluorescence *in situ* hybridization

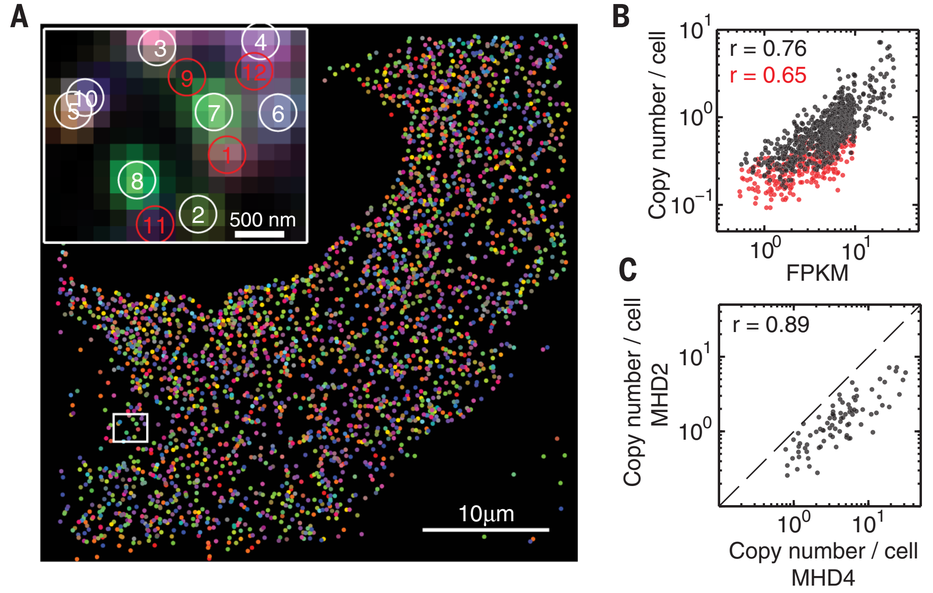
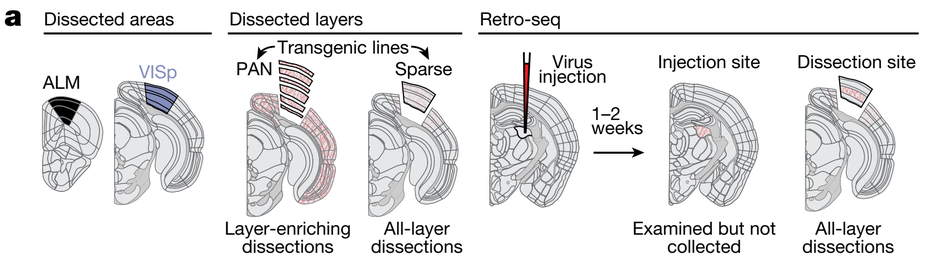
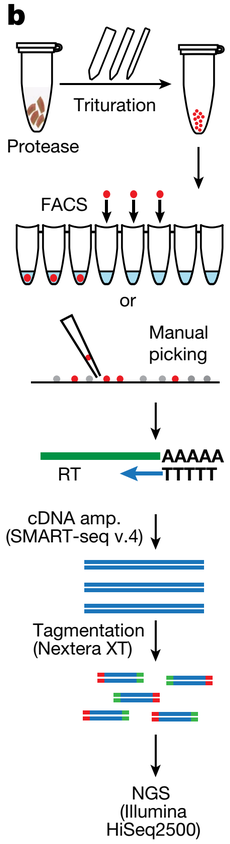


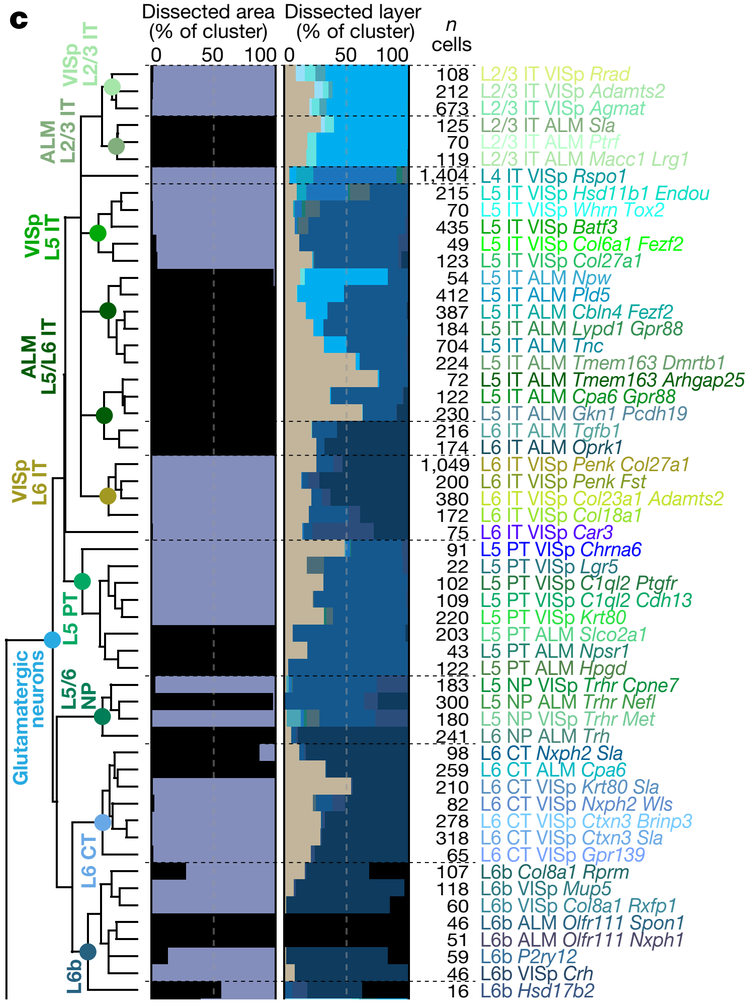
Fig. 5. Simultaneous measurements of 1001 RNA species in single cells by using MERFISH with a 14-bit MHD2 code. (**A**) The localizations of all detected single molecules in a cell colored based on their

(Chen et al. *Science* 2015)

56 Glutamatergic and 61 GABAergic neuron types in visual cortex





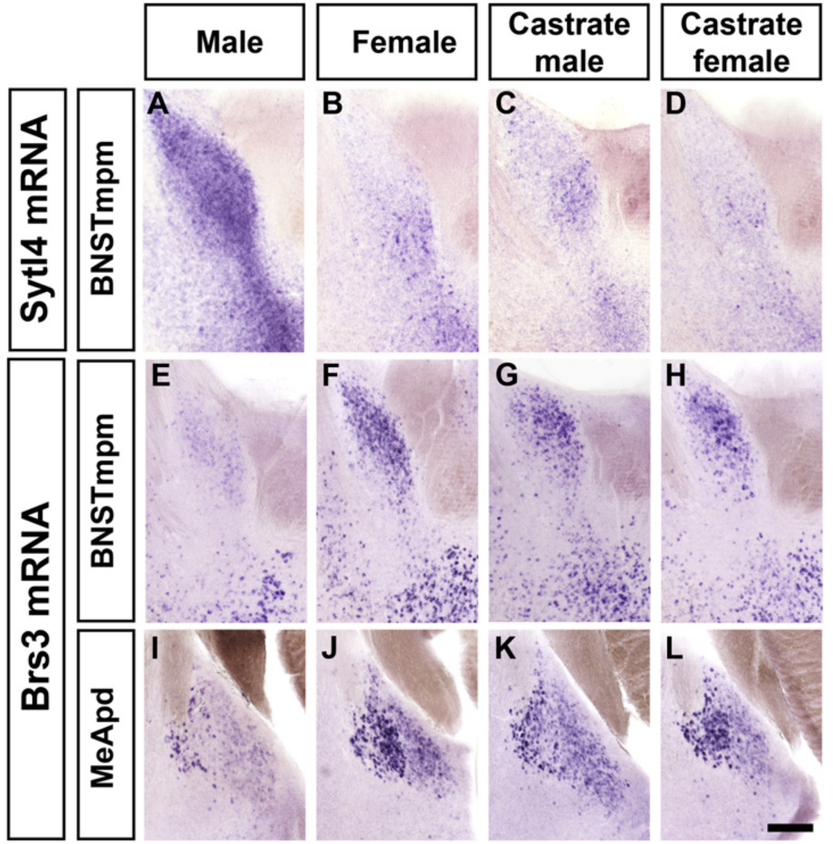


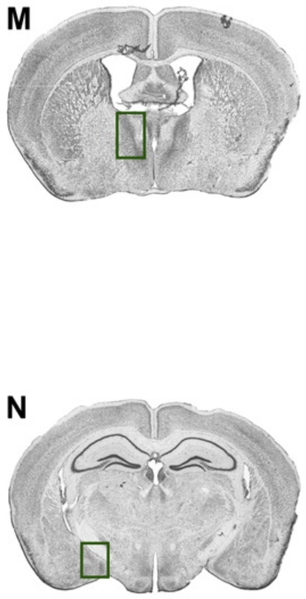
(Tasic et al. *Nature* 2018)

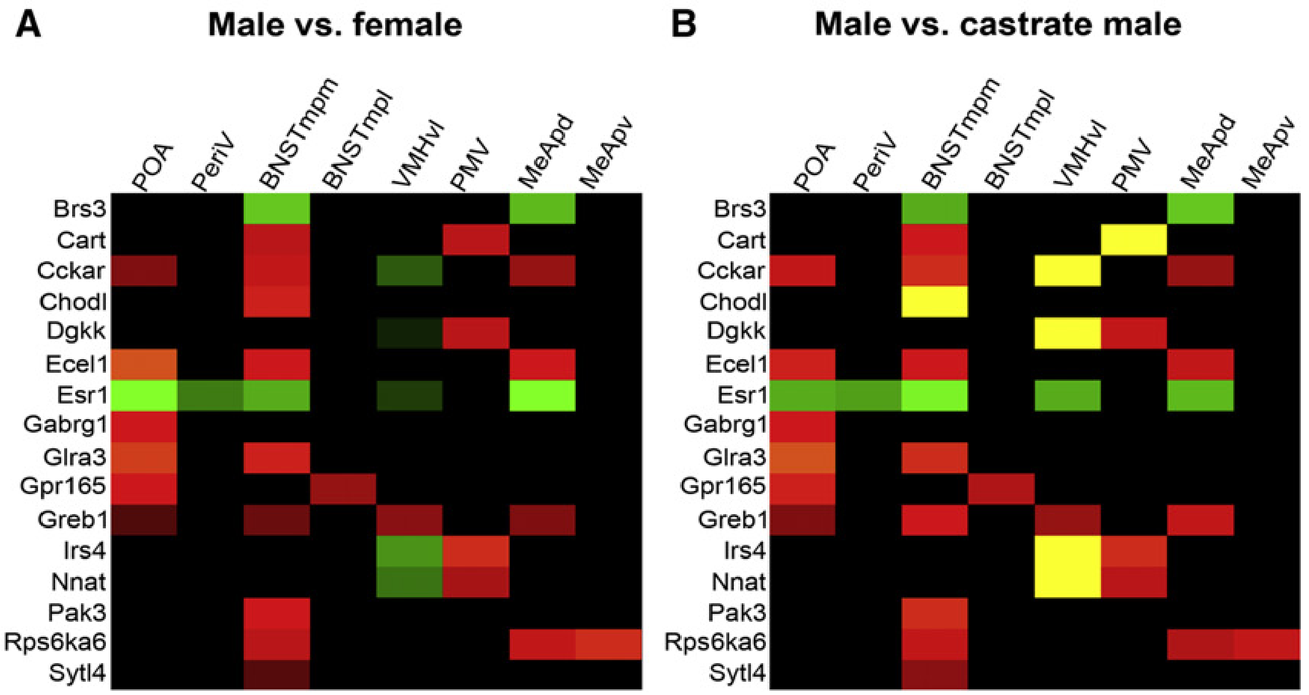
scRNA-seq vs multiplexed RNA FISH

		Throughput	mRNA reads coverage	Major advantage	Major disadvantage
scRNA-seq	Unbiased	med~high (~50 k cells)	highly expressed genes	Easy to perform	Expensive (Typically ~ $10k per experiment ~5000 cells)
Multiplexed FISH	Biased	high (>100k)	low to med expressed genes	Preservation of tissue morphology	Image analysis not trivial

What's the difference between male and female brains?

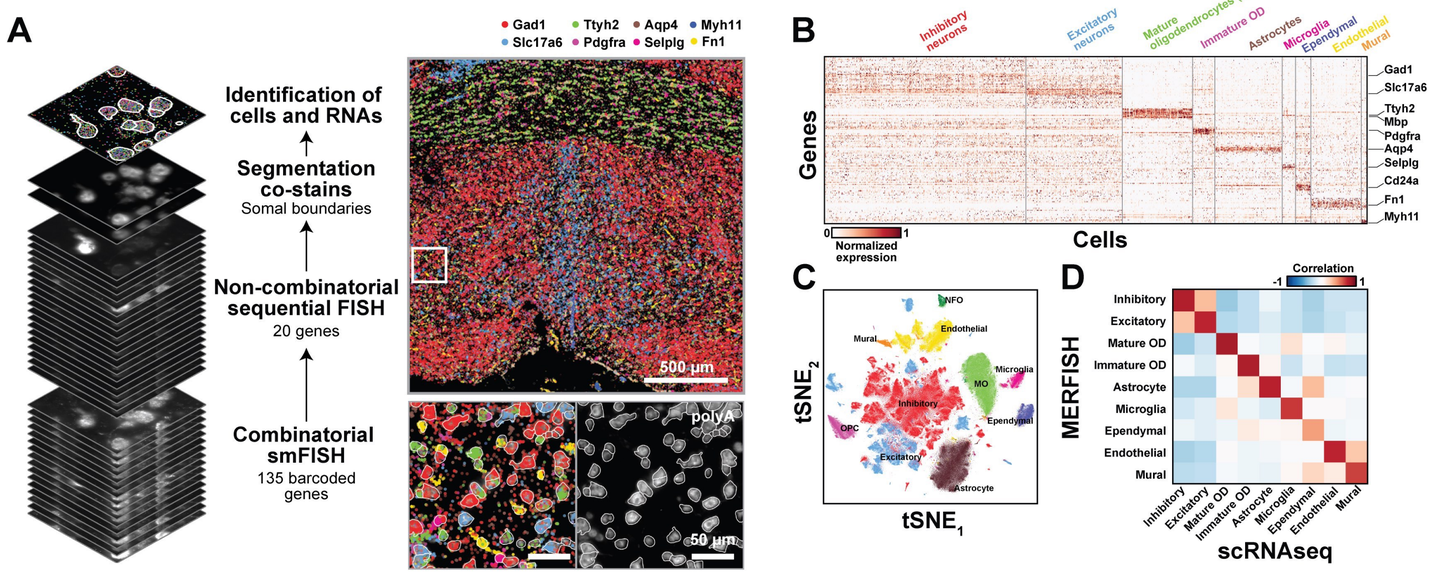






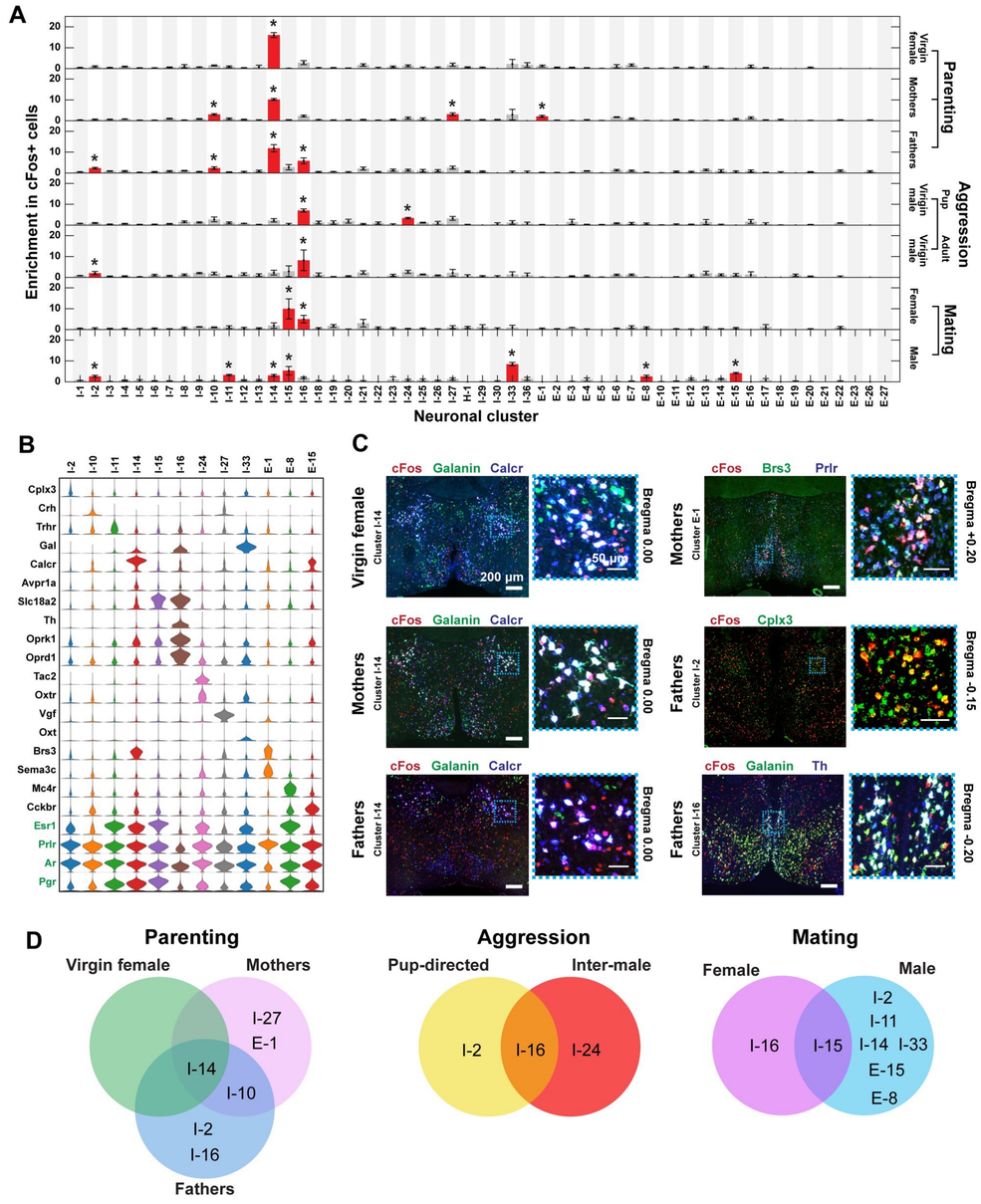
(Xu et al. Cell 2012)

Revealing cell types of social behavior circuits by MERFISH (Multiplexed error-robust FISH)

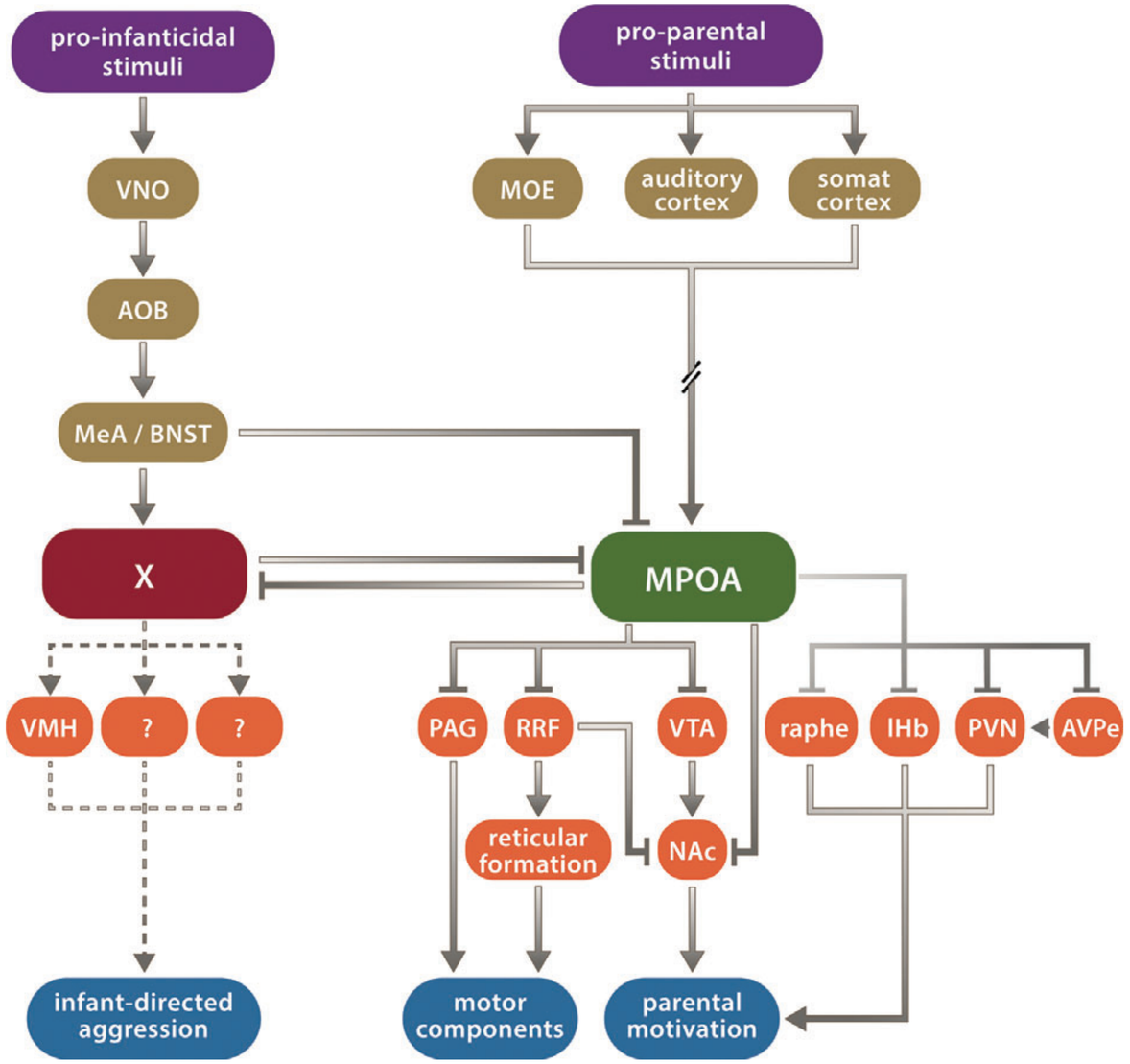


(Moffitt, Bambah-Mukku et al. *Science* 2018)

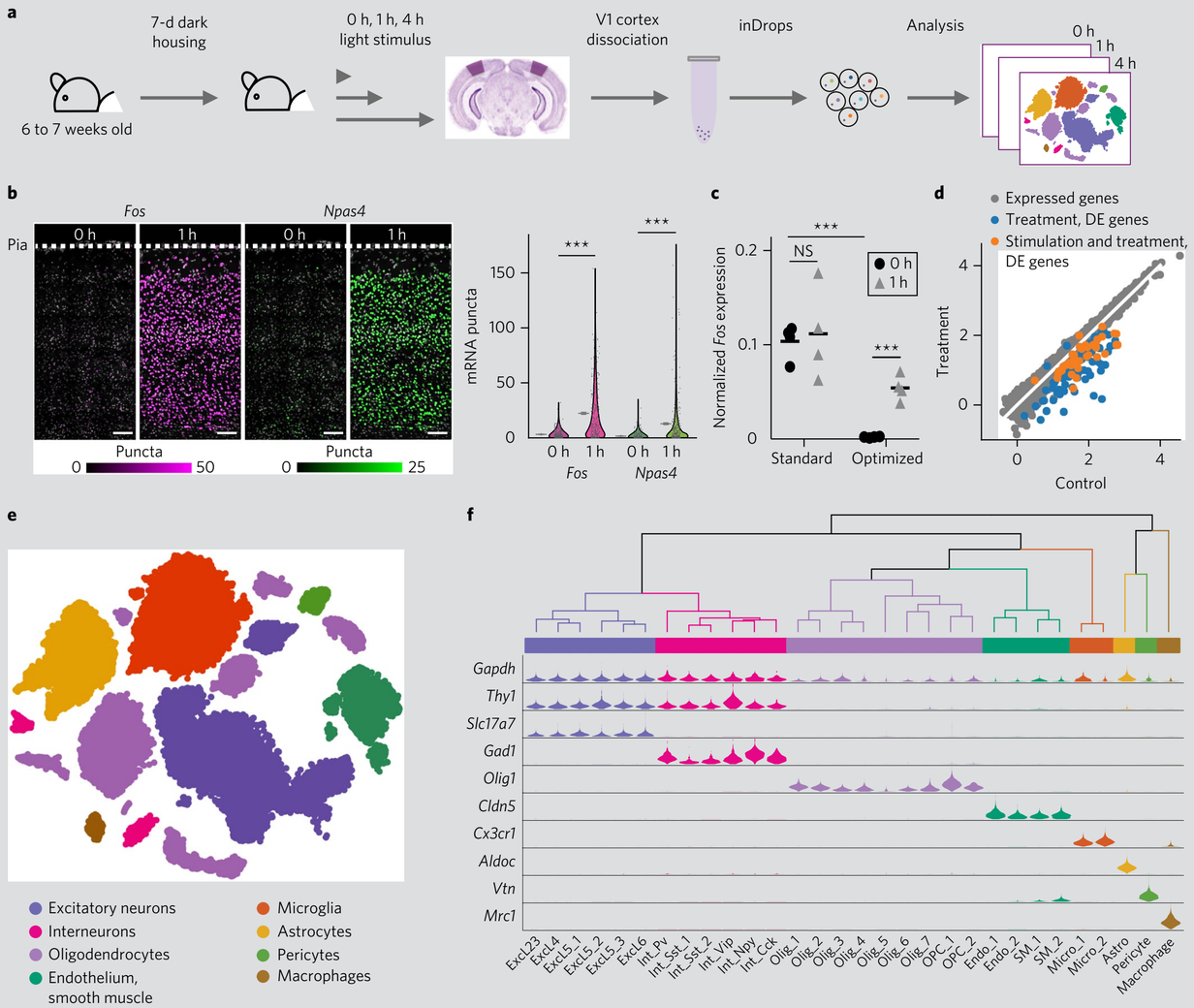
>1 million cells profiled in spatially preserved fashion



Can each neuronal node be genetically defined?



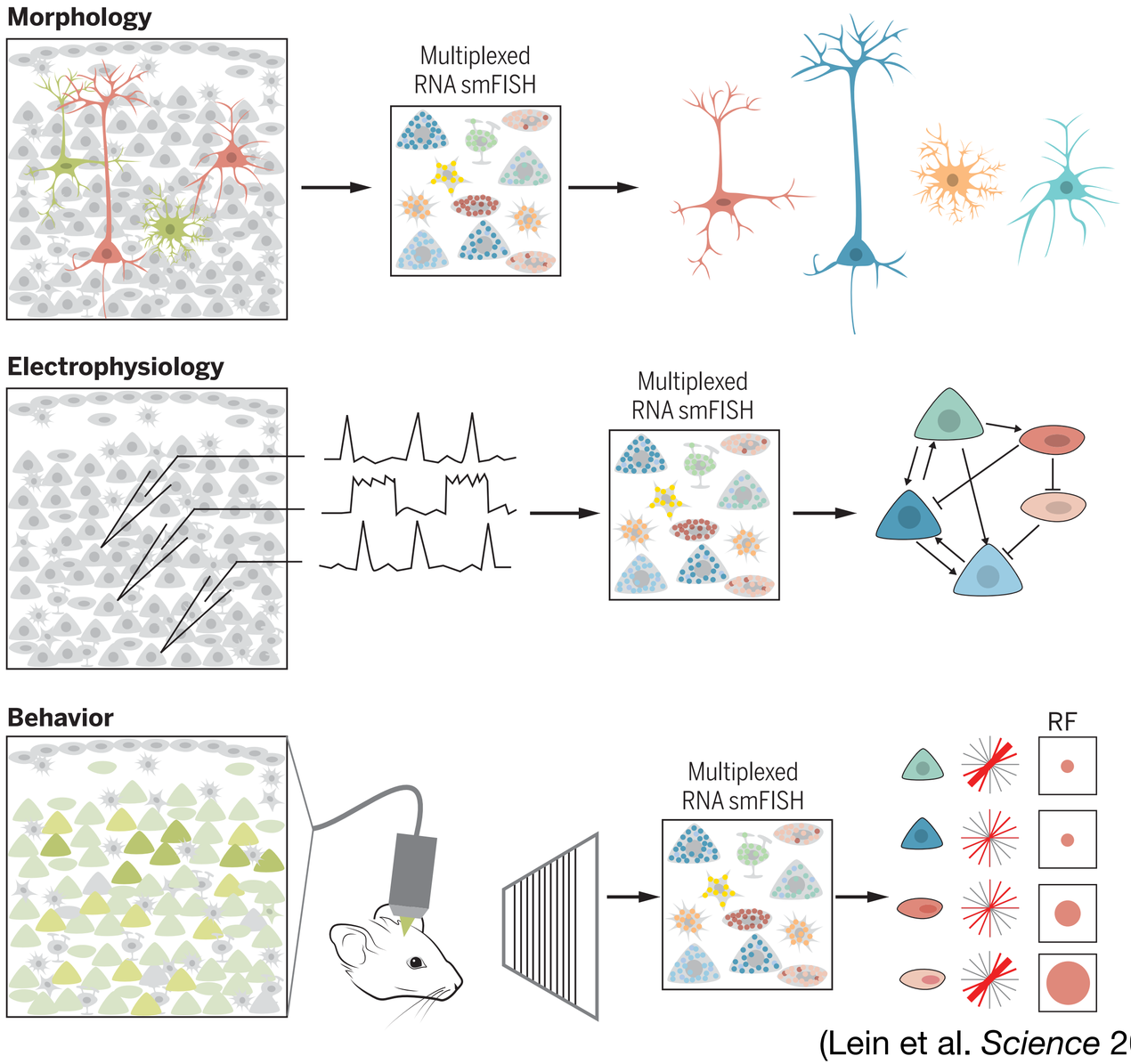
Challenges



(Hrvatin et al. *Nature Neurosci* 2018)

- Expanded classes of cell types: consider activity dependence
- Where should we settle for a meaningful molecular definition of cell types?

Future prospect - multi-level definition of cell types



(Lein et al. *Science* 2017)

Inter-individual variability of social behaviors

Prairie voles are monogamous, but there are cheaters…

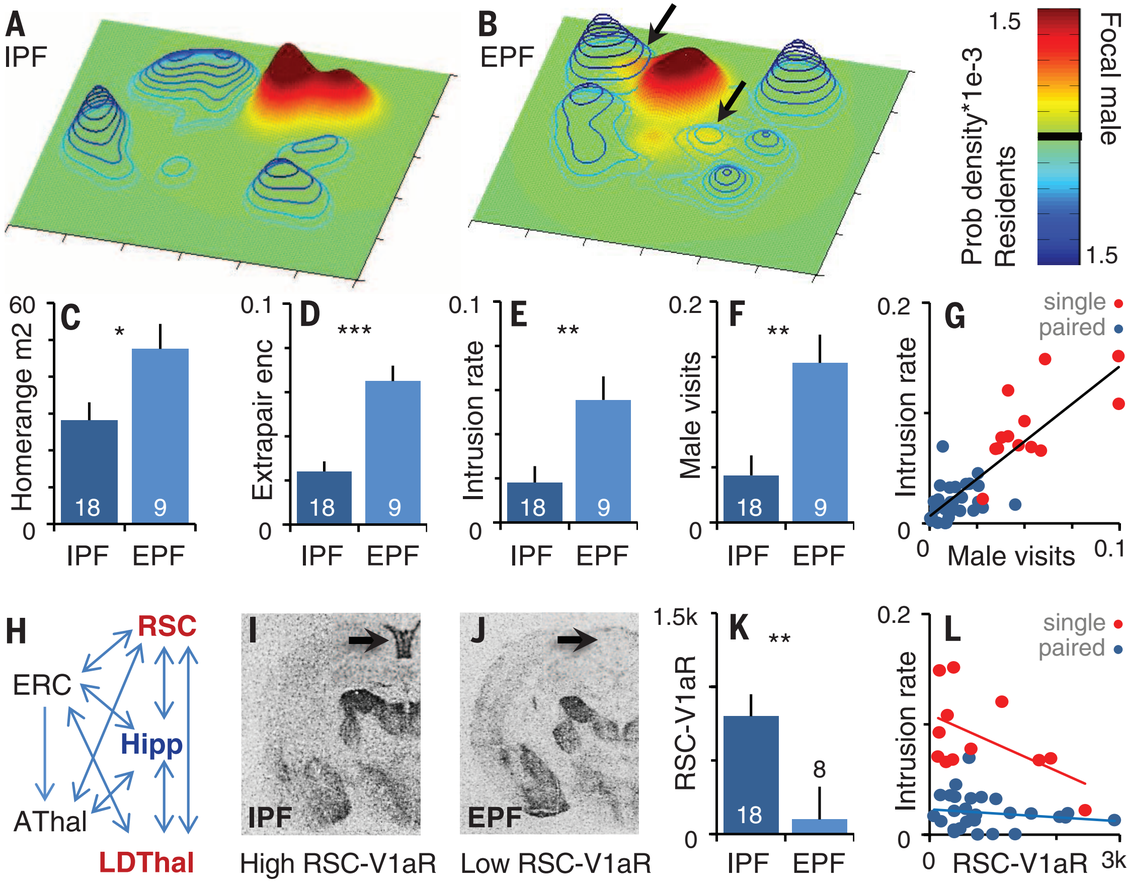


Fig. 1. Male sexual fidelity predicted by patterns of space use, social interaction, and V1aR. (**A** and **B**) Intensity of male space use. The *x* and *y* axes are enclosure dimensions (20 m × 30 m); the height and color of the peaks indicate probability densities. A focal male is indicated as a solid peak; nonfocal males are indicated as blue-contoured peaks. Single males are not shown. Arrows indicate the regions of likely intrusion by the focal male. (**C** to **F**) EPF and IPF males differ in space use. (**G**) Rates of intrusion and of male visitation are correlated. (**H**) Regions of a spatial-memory circuit (*31*) vary in receptors for vasopressin (red) or oxytocin (blue) (*13*, *19*). Abbreviations are as follows: ERC, entorhinal cortex; Hipp, hippocampus; AThal, anterior thalamus. (**I** to **K**) Autoradiograms for V1aR in the RSC. RSC-V1aR abundance (in dissociations per minute per milligram of tissue) predicts sexual fidelity and (**L**) intrusion rate. All bars show mean ± SE. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Analysis of regulatory region of the genome is important!

(Phelps et al. Science 2015)

Summary

- Bottom up approach from molecular front is helpful but has to be done in a meaningful context. Top-down and bottom-up approaches need to be used in combination.

- Immense cellular diversity in the brain
  - Unique set of markers
  - The ways in which cells respond to action potential

- Currently, there isn’t a lot of functional studies linking cell diversity and behavior

Module 3: Social and Affective behaviors

Learning goal:

1) Why is it important to study natural behavior of animals to understand the brain?

2) What are the constraints of innate behaviors?

3) How are innate behaviors implemented, at behavioral, circuit and molecular/genetic levels?