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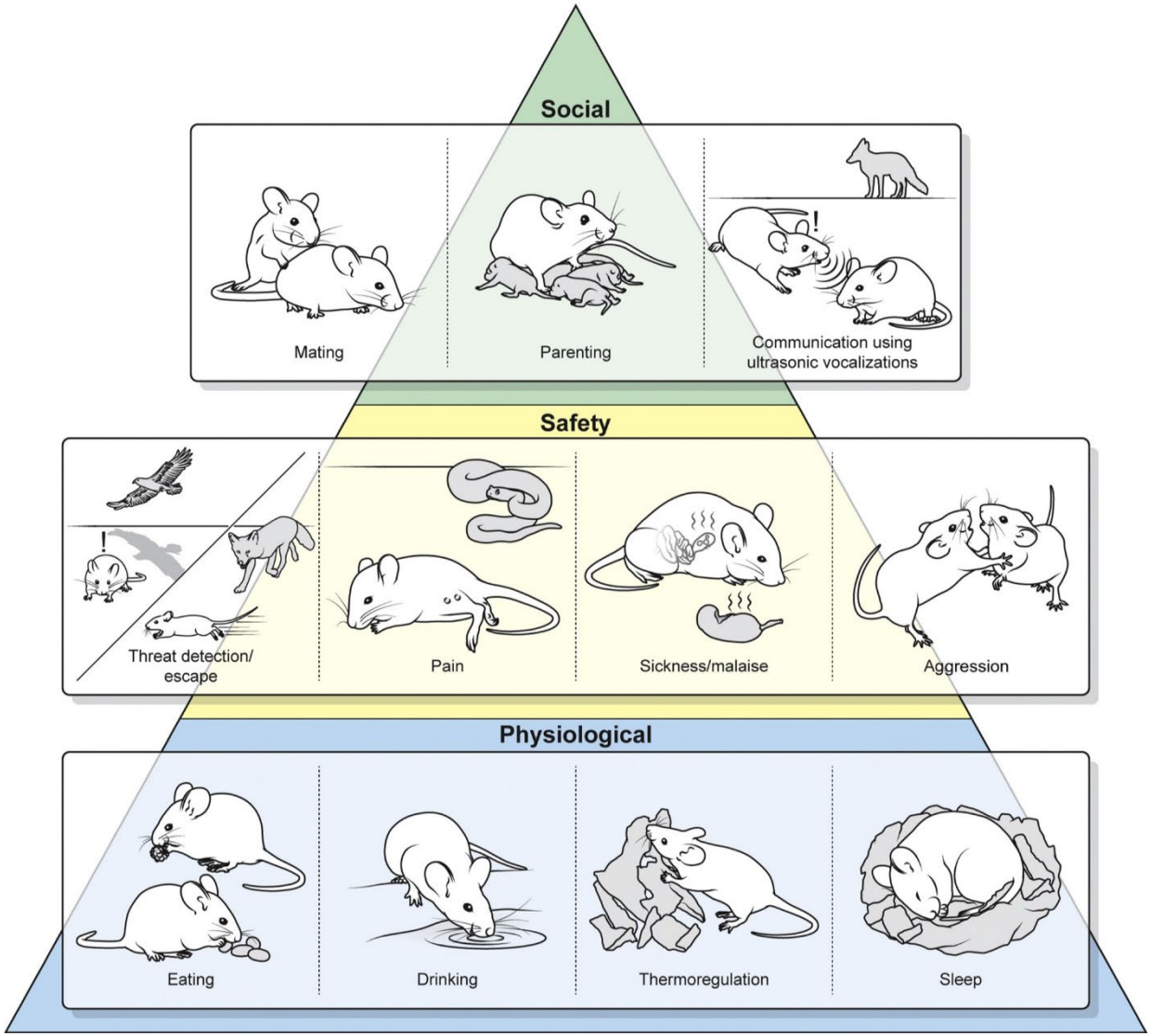
Course "Neural Circuits of motivated behavior"

Unit 7,
Internal Body States: Thirst

Ralf Schneggenburger

Thursday 03.04.2025
(Week 7)

Different needs of animals



"Maslow's hierarchy of needs"

after Sutton & Krashes 2020

Homeostasis

= maintaining a stable internal milieu
(e.g. blood glucose, **blood electrolytes and water**, body temperature)

- term coined by Walter Cannon ~ 1930
(also French physiologist Claude Bernard, 19th century)

- regulated value (e.g. blood glucose level)
- **setpoint** (built-in goal value)

● mismatch / error?

IF YES => "**drive state**"

=> triggers **correction response**

- in case of body energy state, body electrolyte / water balance:

hunger => food seeking, eating **Thirst** => water seeking, drinking

"ingestive motivations"

=> negative feedback, that physiological reality comes back to set-point

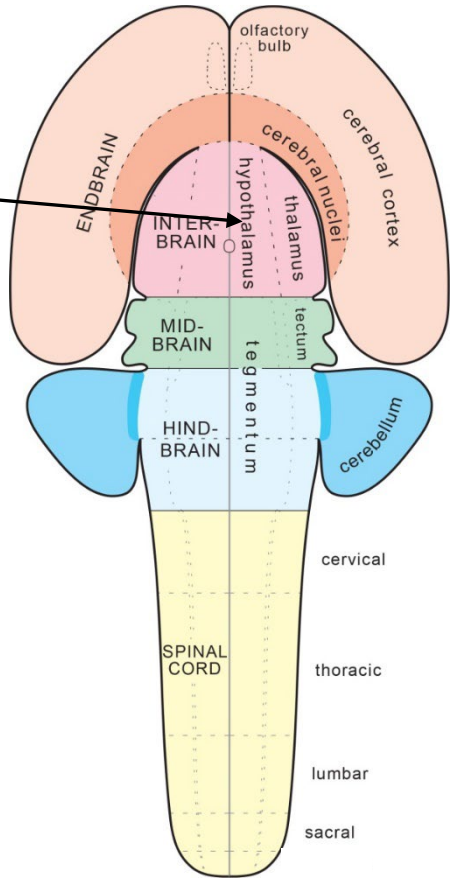
after Berridge, 2004

1) Introduction to the Hypothalamus

Anatomy, and function overview

Hypothalamus

(part of the diencephalon, interbrain)



brainstem

The hypothalamus receives information from the outside of the body (exteroception)
and the inside of the body (interoception),
and is a prime regulator of physiological needs of the body (enables "homeostasis")

The hypothalamus homeostatically (and non-homeostatically) regulates these function, via three "motor-like" outputs:

• via the somatic motor system
(e.g. Temperature regulation: go to a warmer place; shivering)

• via the autonomic nervous system
(e.g. Temperature regulation: sweating)

• via the neuroendocrine system
hypothalamic - pituitary axis, HPA
release of "releasing hormones", which stimulate release of other hormones, in the body.

Inputs to the hypothalamus

important inputs:

- olfactory bulb
- **prefrontal cortex**
- **cingulate gyrus**
- **septum**
- thalamus
- **hippocampus**
- **amygdala**
- retina *
- nucleus of the
- solitary tract (NTS)

many inputs from **limbic system** brain areas!

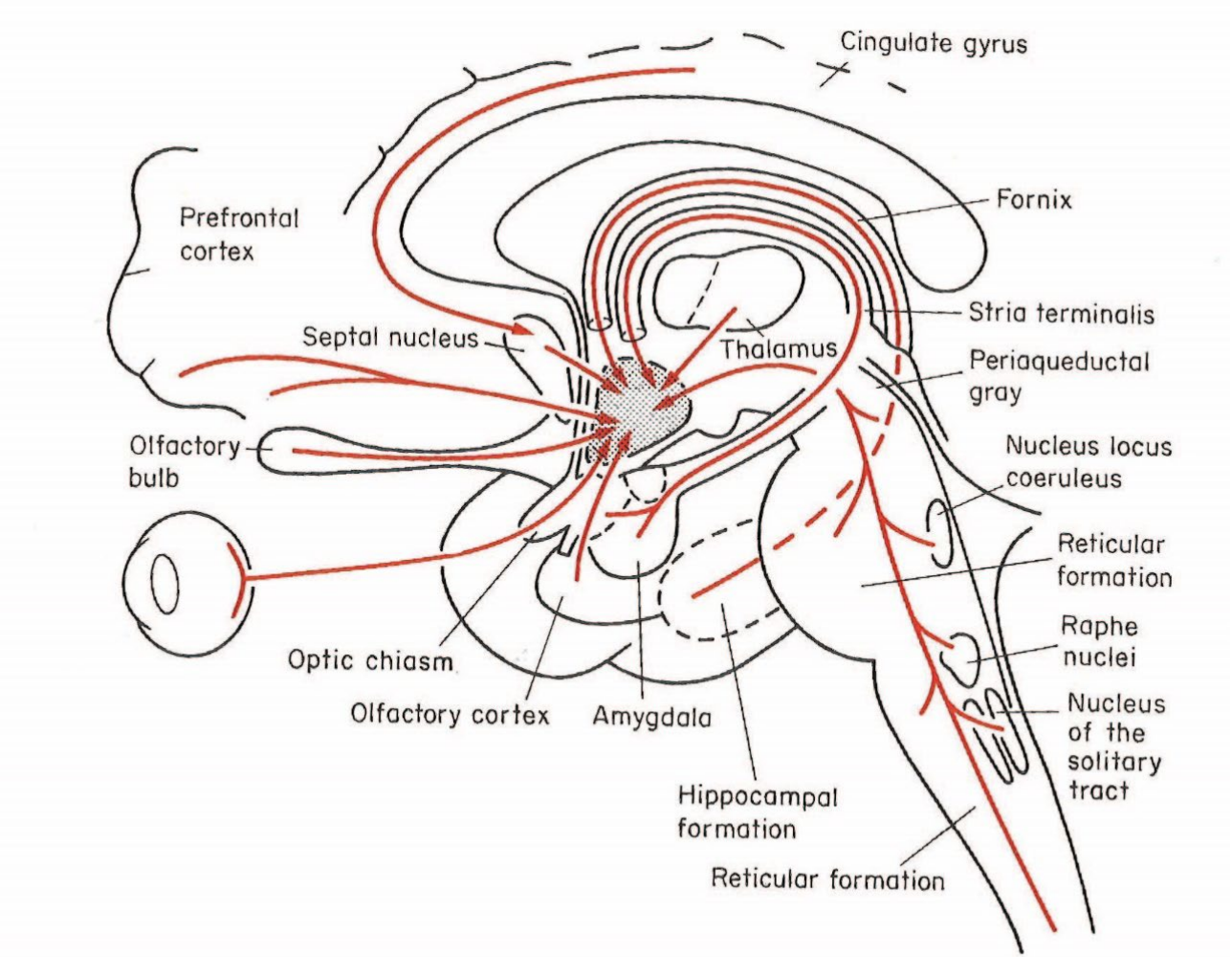
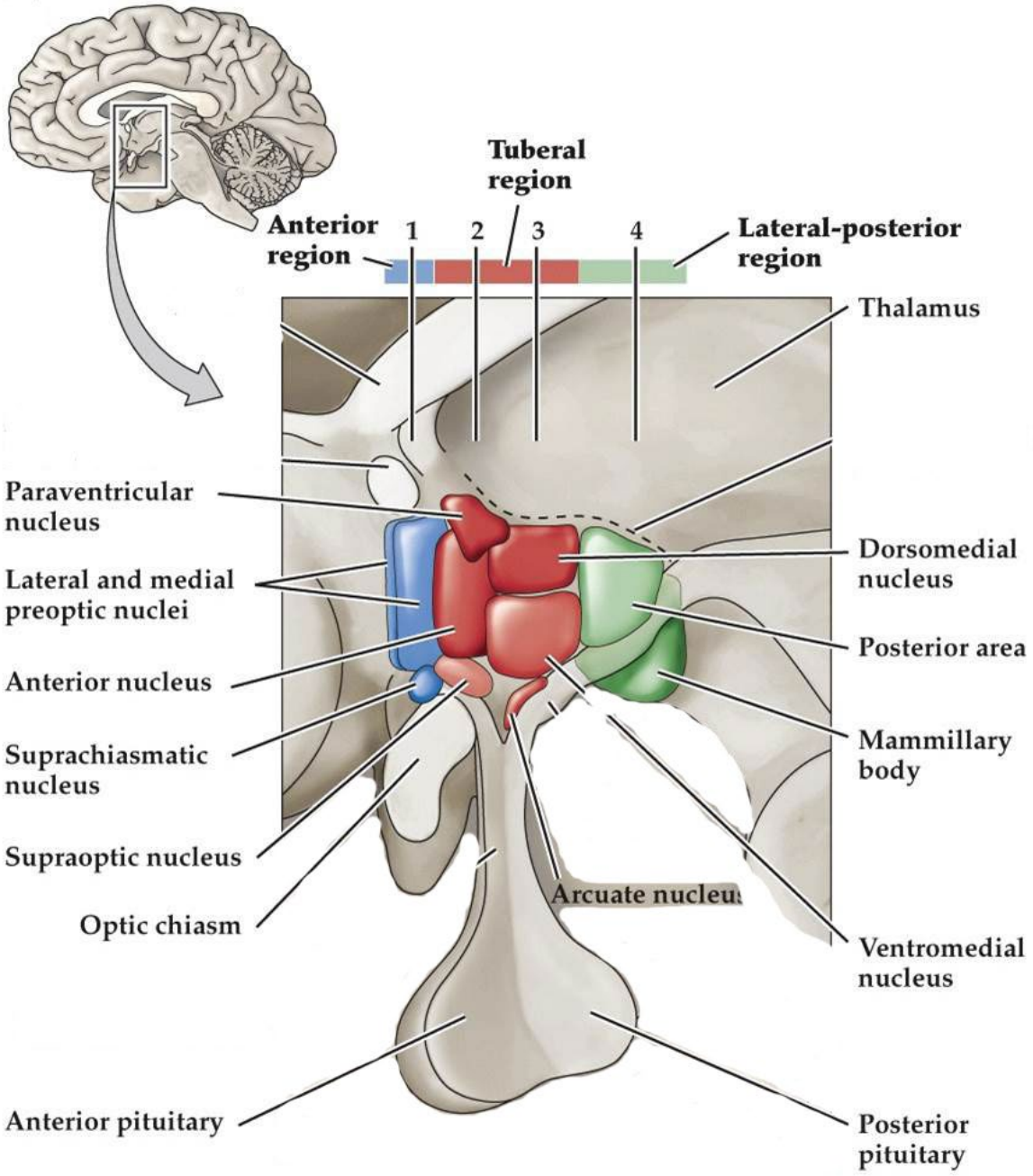


Fig. 15.2. *Main afferent connections of the hypothalamus.* Arrows indicate the direction of impulse conduction.

Brodal, Fig. 15.2



Hypothalamus:
- here: side-view (in human)

- located on ventral side of diencephalon ("below" thalamus)

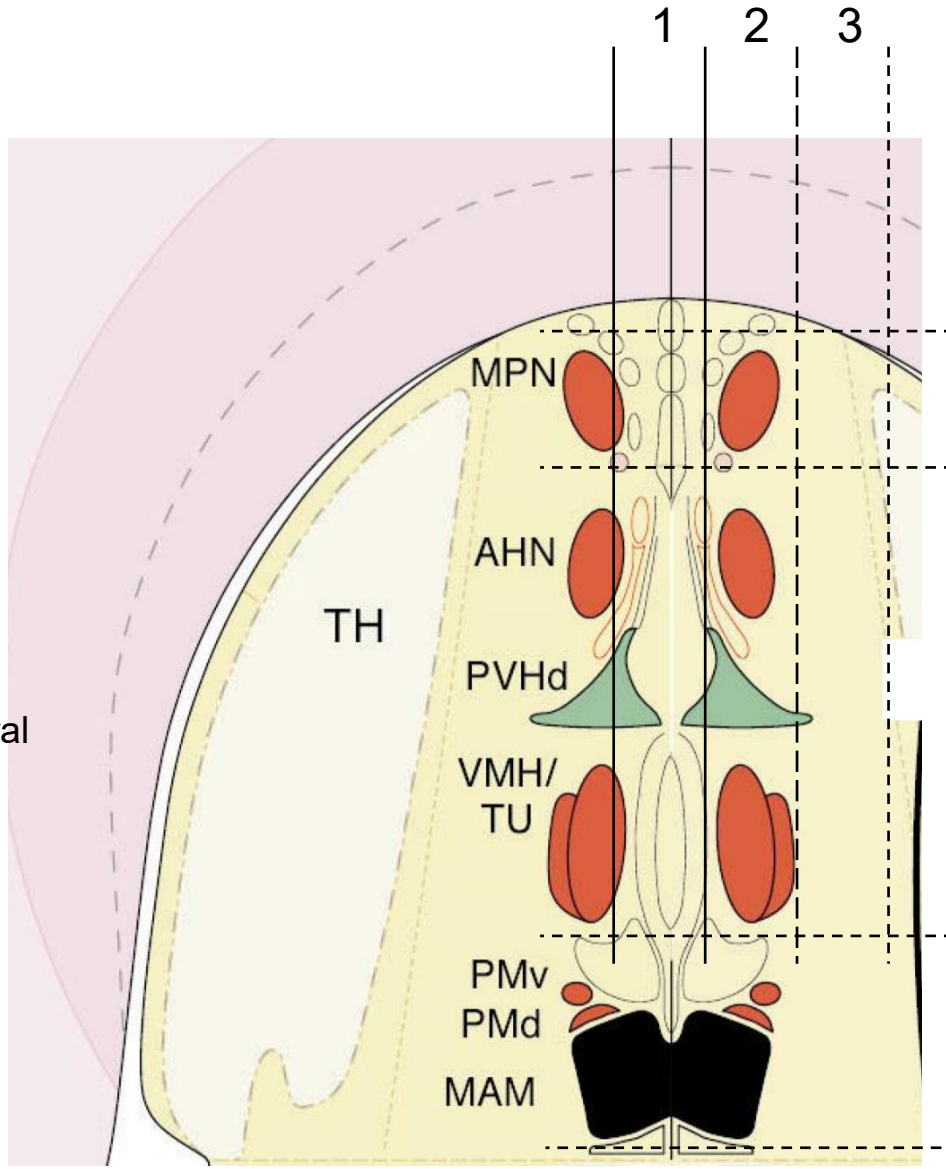
- continuous with hypophysis (=pituitary)

- many different sub-nuclei ...
- with different functions

Purves Box 21.A

Divisions of the Hypothalamus

from medial -to- lateral:
1 periventricular region
2 **medial** region
3 lateral region



important nuclei of medial region:

anterior

MPN or **MPON**:
medial preoptic nucleus

AHN:
anterior hypothalamic nucleus

tuberal

PVH:
periventricular nucleus of hypo-thalamus

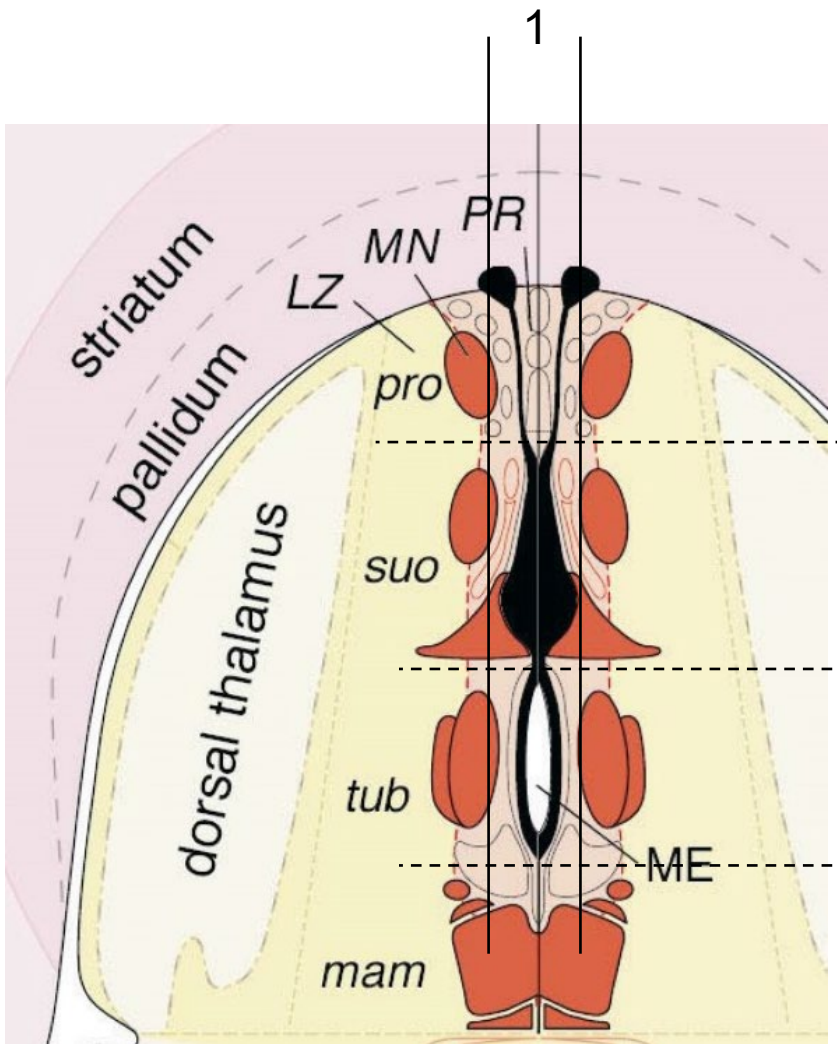
VMH:
ventromedial nucleus (of h.)

mammillary (posterior)

PMv, d:
premammillary nucleus, ventral, dorsal part

Swanson 2000, Fig. 10

Layout of hypothalamic nuclei along rostro-caudal, and medio - lateral axes



dark red:
main nuclei in medial region:

preoptic (pro / MPON)

supraoptic or anterior (AHN)

PVH

VMH

Mammillary

black: *neuroendocrine motor zone*
(in periventricular region)
light red: periventricular region (PR)

ME:
Median eminence

Swanson 2000, Fig. 8

Outputs of the hypothalamus

outputs to

- **prefrontal cortex**
- **septum**
- thalamus
- **amygdala**
- **hippocampus**

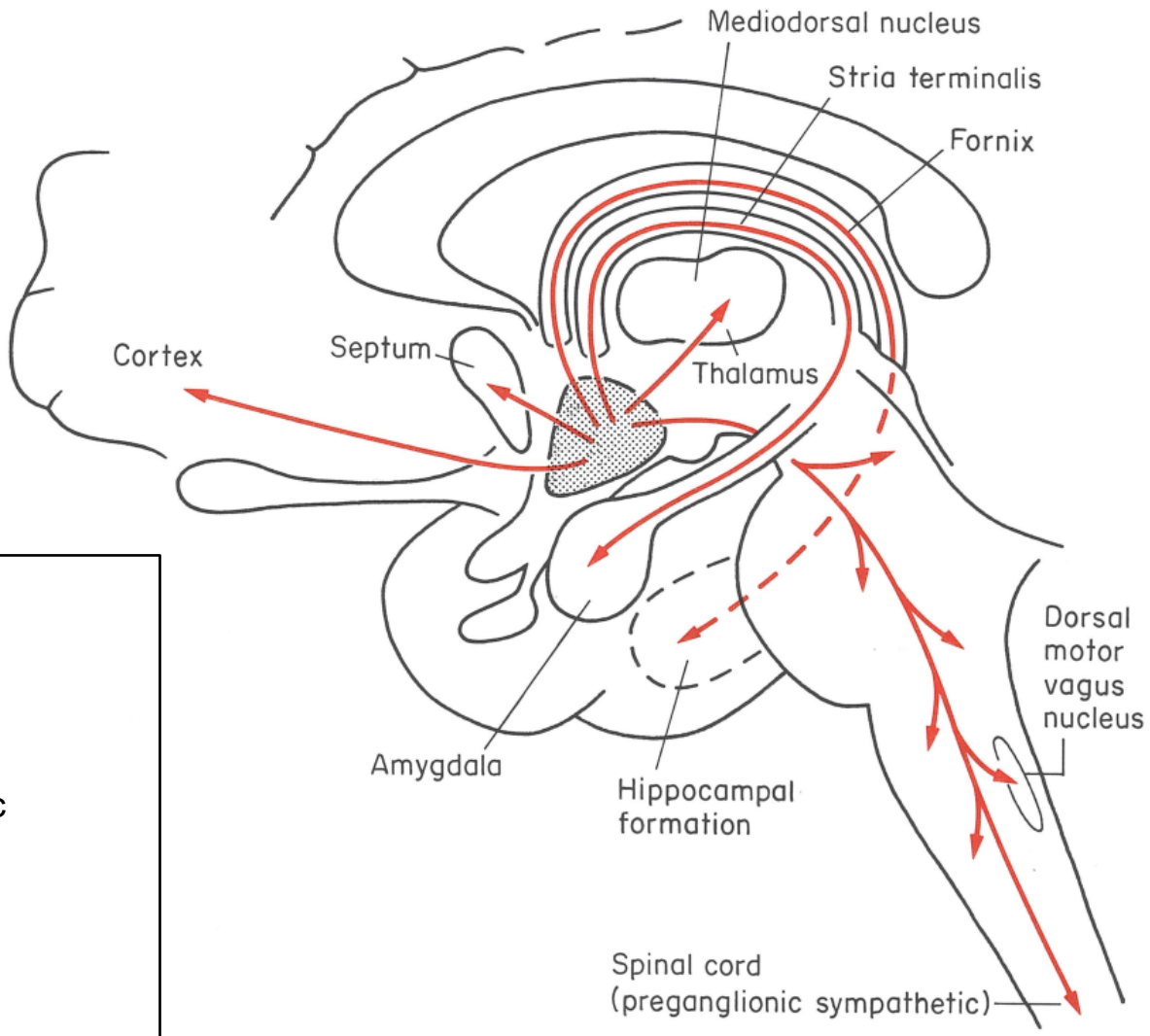
... reciprocate the inputs !

other important outputs

- dorsal motor nucleus of vagus
- preganglionic sympathetic neurons in spinal cord

=> output to **autonomic nervous system**

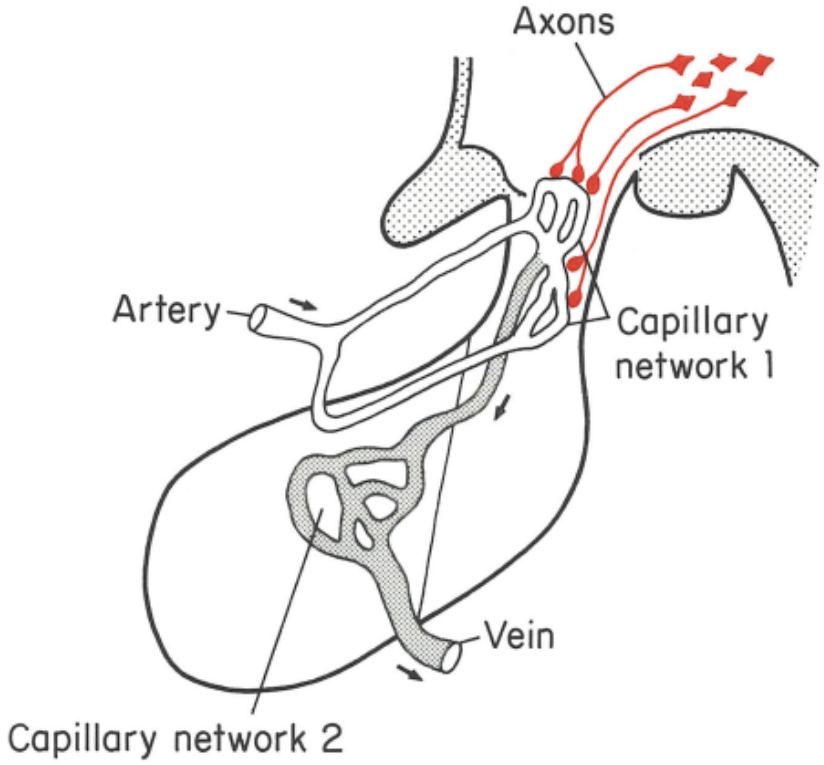
- periaqueductal grey (PAG)
=> motor outputs (freezing, escape, aggression) see unit 6 !



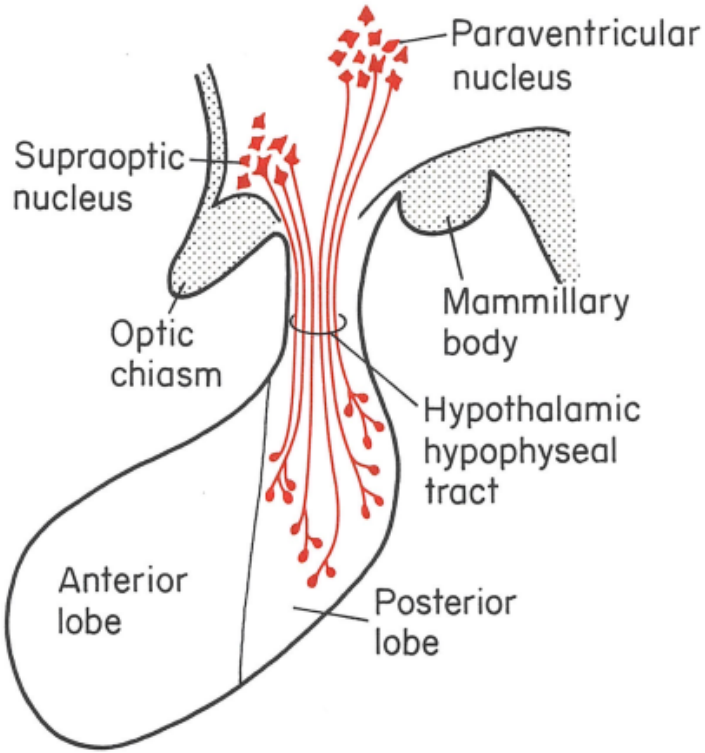
=> These outputs cause "**motor actions**" via the **somatic motor system** and **autonomic nervous system**

"Neuroendocrine" function of the hypothalamus:
two principal connections to i) the **anterior** and ii) the **posterior hypophysis**

a) Portal vein system carries "**releasing factors**" produced by hypothalamic neurons to the **anterior lobe** of the hypothesis.

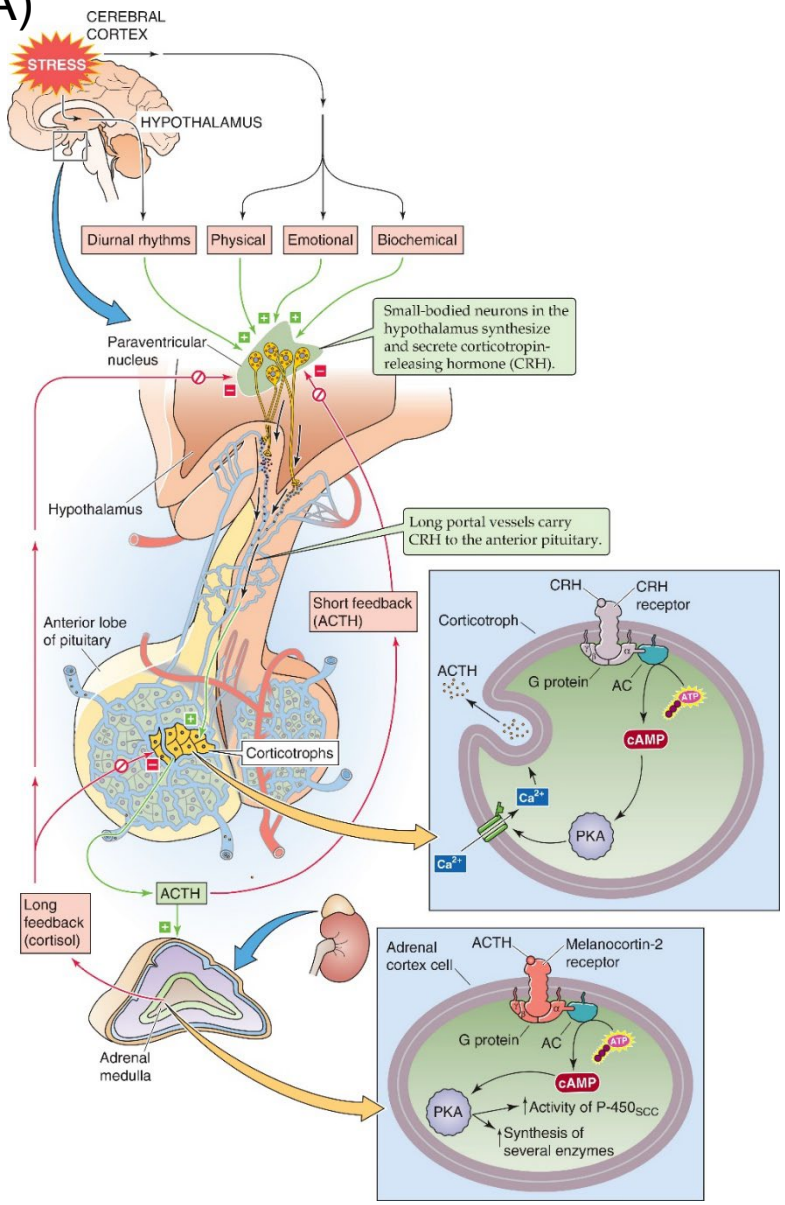


b) Long axons of some hypothalamic neurons project to the **posterior lobe**r, where they releases hormones directly into the bloodstream



Brodal, Fig. 15.5

One example of a Neuroendocrine function of the hypothalamus: Control of the release of stress hormone (cortisol) in the Hypothalamic-pituitary -adrenocortical axis (HPA)



CRH acts via the **portal vein** onto **corticotroph cells** in the anterior hypophysis

CRH
Corticotropin
Releasing
Hormone

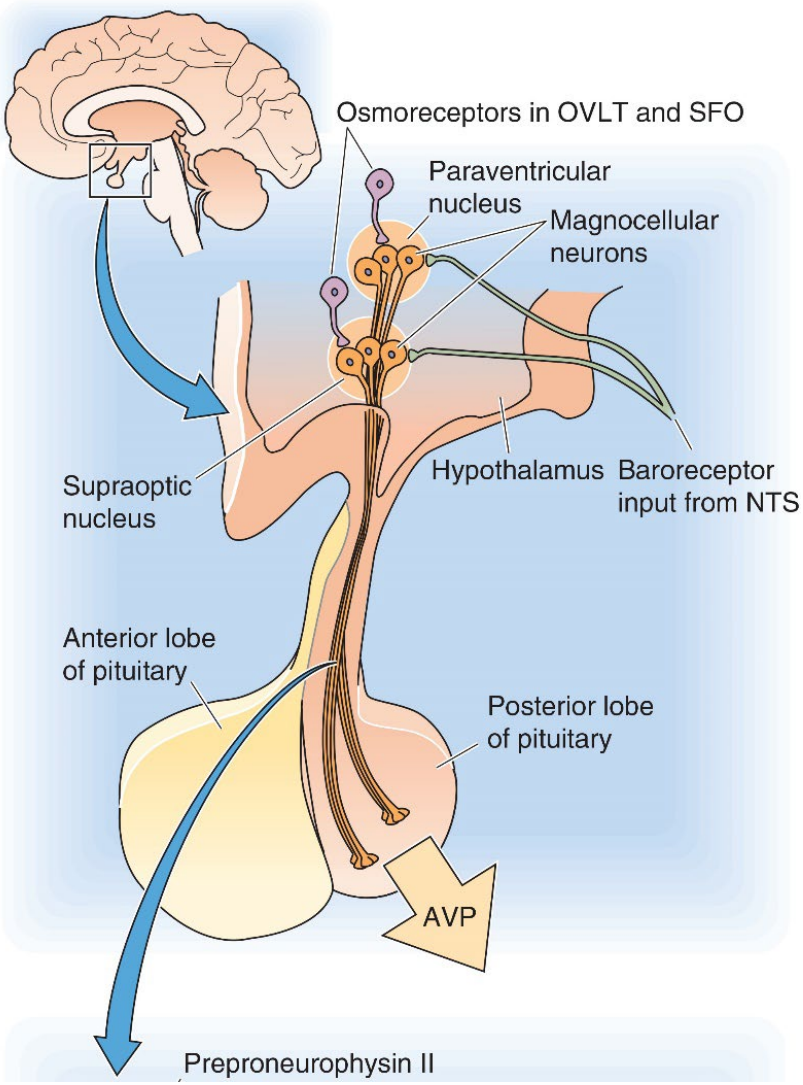
ACTH
Adreno
Corticotropin
Hormone

cortisol
(released from adrenal cortex)

Boron, Fig. 49.3

Another example for a neuroendocrine function of the hypothalamus:
An increase in blood osmolality caused AVP release

Arginine-vasopression (AVP):
- produced by magnocellular neurons in the PVN.
- These neurons send their axons into the posterior hypophysis
- There, AVP is released from nerve terminals into the bloodstream



Boron Fig. 40-8

=> AVP acts in kidney (↑ H_2O re-absorption)

Hypothalamus – integrative function for 6 main physiological needs:

1) Blood pressure and electrolyte composition

- by controlling drinking, salt appetite
- Paraventricular nucleus
- magnocellular neurons send their axons into neurohypophysis (= posterior hypophysis); axon terminals release Vasopression (ADH) (+Oxytocin)

2) Energy metabolism

- monitoring blood glucose, controlling hunger + feeding behavior, metabolic rate)

3) Reproduction, sexual & mating behavior

- sex hormones in females; hormone control of mating, pregnancy, lactation)
- via controlling FSH + LH release from adenohypophysis (Follicle-Stimulating Hormone + Luteinizing Hormone)
- via Oxytocin release in neurohypophysis

4) Regulation of **body temperature**

- thermoregulatory behavior (seeking warmer/colder environment; shivering)

5) Defensive Behavior

- Hypothalamus regulates stress response, and fight-or-flight responses
- regulates behavior directly
- on more long-term, via controlling release of adrenal stress hormones, HPA axis
- Paraventricular-, anterior- , dorsal premammilary Nuclei,
  and LH lateral hypothalamus.

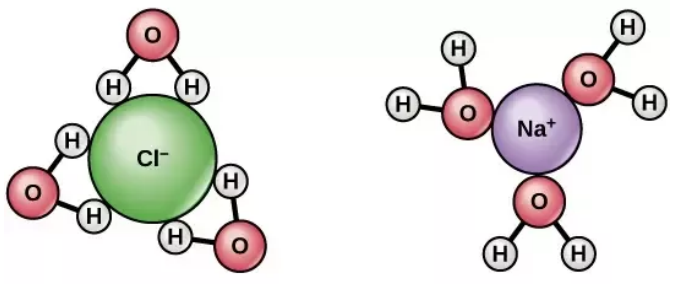
6) Circadian rythm; sleep-wake cycle

- "circadian clock" in suprachiasmatic Nucleus
- levels of arousal when awake (Lateral hypothalamus)

2) Thirst, Regulation of blood volume and blood osmolality

2a) Physiological bases

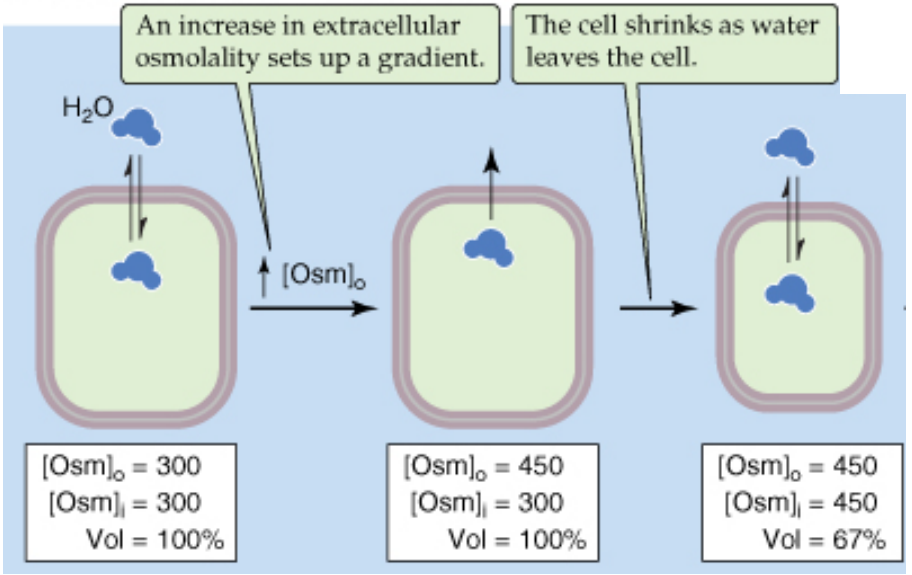
Water is life:
Life, as we know it, happens in aqueous solutions



The physiological basis of body fluid regulation (next ~ 15 slides) follows:

Boron, Medical Physiology:
Chapter 40:
"Integration of Salt and Water balance"
(Giebisch, Windhager & Aronson)

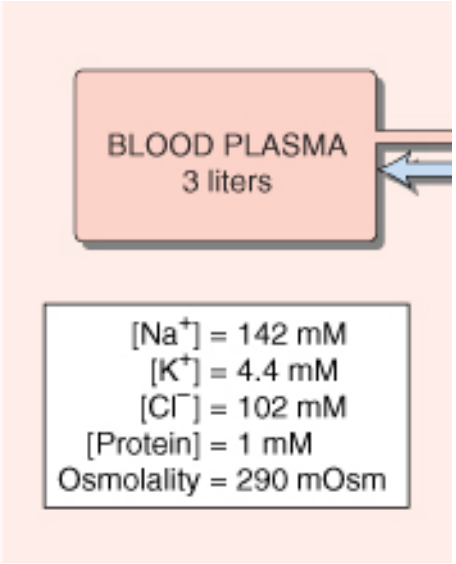
On Moodle

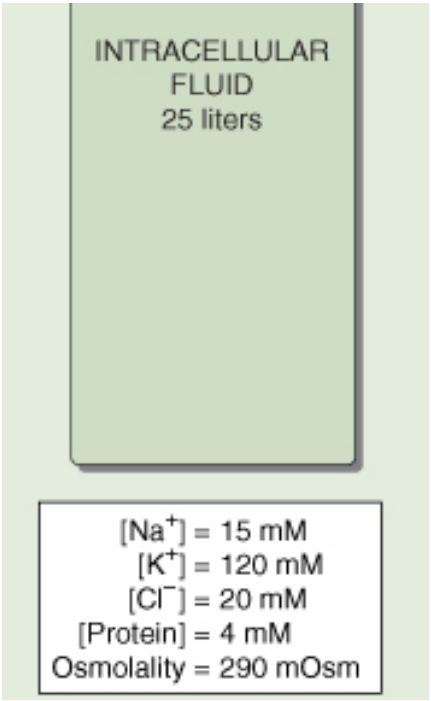


Boron Fig. 3-15

- H_2O can easily pass membranes (water channels!)
- An increase in extracellular ion concentration (osmolality)
  … causes cell shrinking

=> Water and ion content in body fluids need to be carefully balanced!





- Remember:

Extracellular:
high [Na^+], low [K+]

//

Intracellular:
rel. low [Na^+], high [K^+]

- Also:
osmolality tightly regulated (290 mosm) and SAME intra- and extracellularly !

(blood plasma:
surrogate of extracellular fluid, ECF)

Perspiration:
Sweat fluid is somewhat hypo-osmolar: ~ 40 - 60 mM [Na^+], ~ 40 mM [Cl^-]

thus Sweating (~ 2 liters/ hour in extreme conditions)

=> ↓ in body water volume / blood **volume**

=> ↑ in blood **osmolality**

=> Drives strong feeling of "thirst": search for - and consume water

Also: Hemorrhage (strong bleeding):

=> ↓ in blood **volume**

=> Also drives "thirst"

There are two variables that are monitored in fluid control:

1) Control of extracellular fluid **volume**

2) Control of water content
(extracellular osmolality) - *below*

Feedback loop controlling the effective circulating **volume**

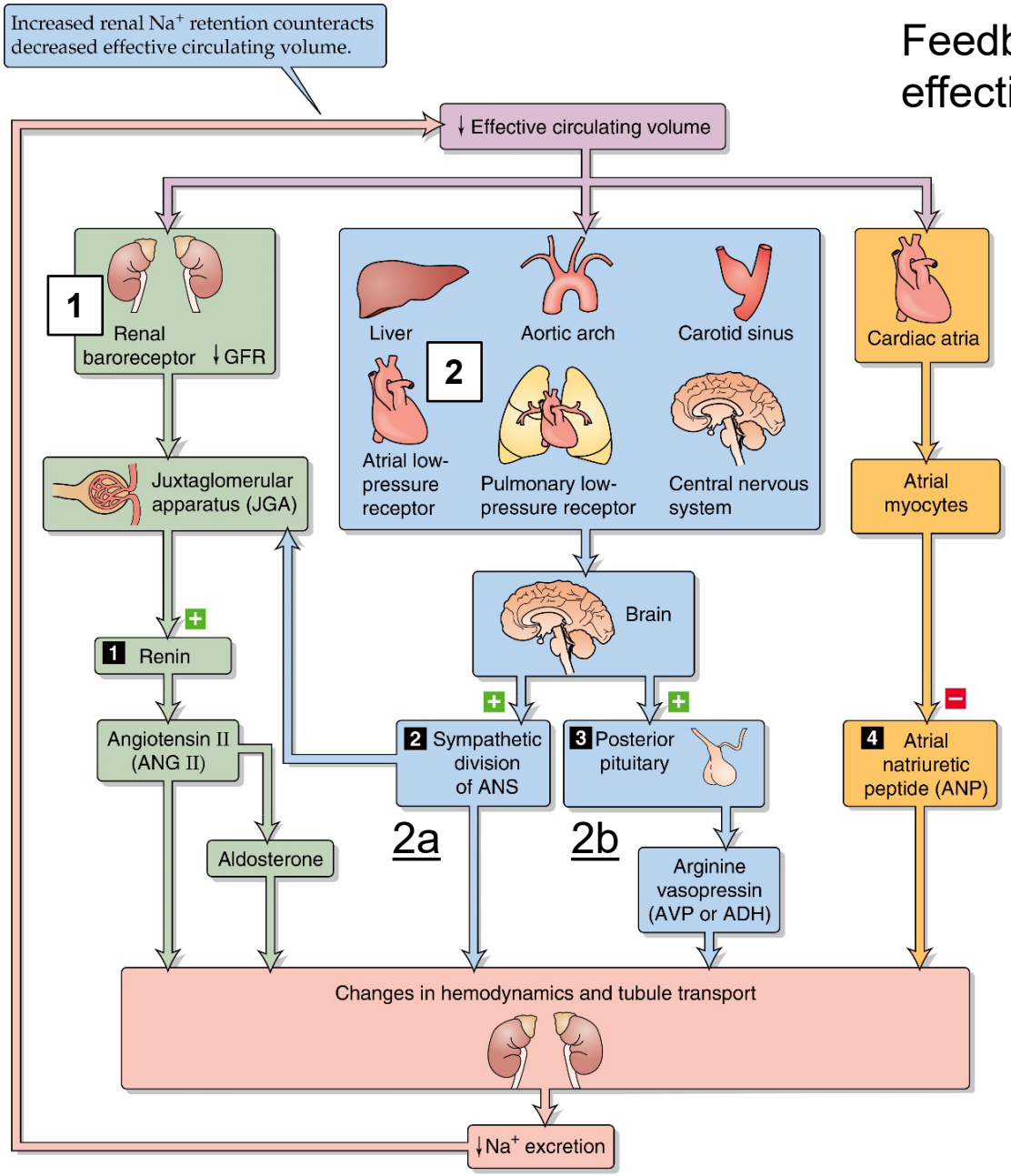


Figure 40-2 Feedback control of effective circulating volume. A low effective circulating volume triggers four parallel effector pathways (numbered *1* to *4*) that act on the kidney, either by changing the hemodynamics or by changing Na^+ transport by the renal-tubule cells. ANS, autonomic nervous system.

Boron Fig. 40-2

Sensors (baroreceptor):

1) afferent arterioles of kidney
2) Atrial low-pressure receptors

These sensors generate four distinct hormonal - or neuronal signals:

1) Kidney: Renin - angiotensin system

2a) Low-pressure baroreceptors
→ afferent neurons (N. vagus) → medulla → Sympathetic div. of ANS
→ ↓ blood renal blood flow (= ↓ urine production)

2b) Low-pressure baroreceptors
→ afferent neurons (N. vagus) → Nucl. Tractus solitarius (NTS, in medulla) → →
posterior pituitary → release of Arginine-vasopression / antidiuretic hormone (AVP/ADH)
into blood stream (= ↓ urine production)
(2b: *only after large ↓ in circulating volume*)

4) ***less*** release of Atrial natriuretic peptide fr. atrial myocytes (ANP, promotes Na^+ excretion)
→ ↓ excretion of Na^+ → conserves water & volume

2) Control of water content (extracellular **osmolality**)

Two limbs in the control of body fluid osmolality:

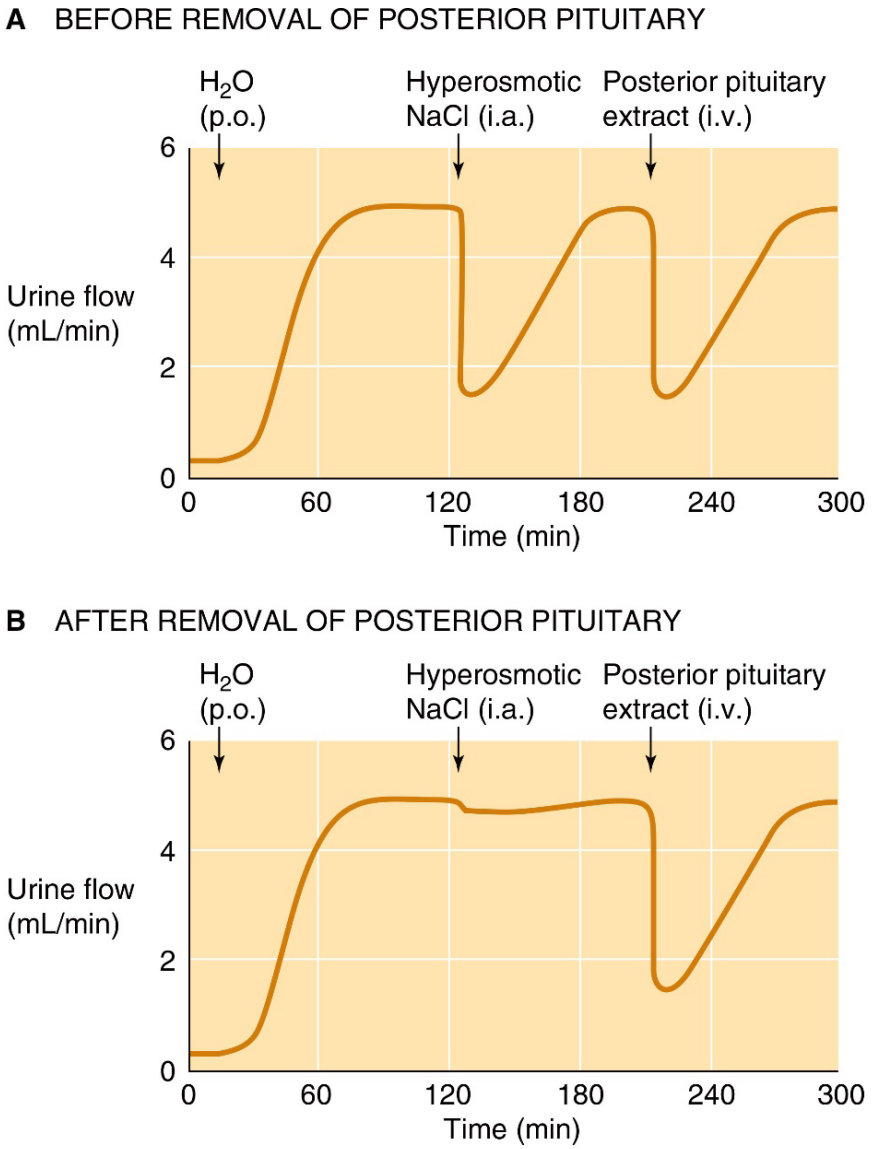
a) Control of water excretion (kidneys)

b) Thirst (↔ oral intake of water)

a) Control of water excretion (kidneys)

Injecting hyperosmotic NaCl solution into carotid artery of a dog

=> decrease in urine production that *depends* on posterior pituitary



Also:

- effect of *posterior pituitary extract* injected intravenously suggests:

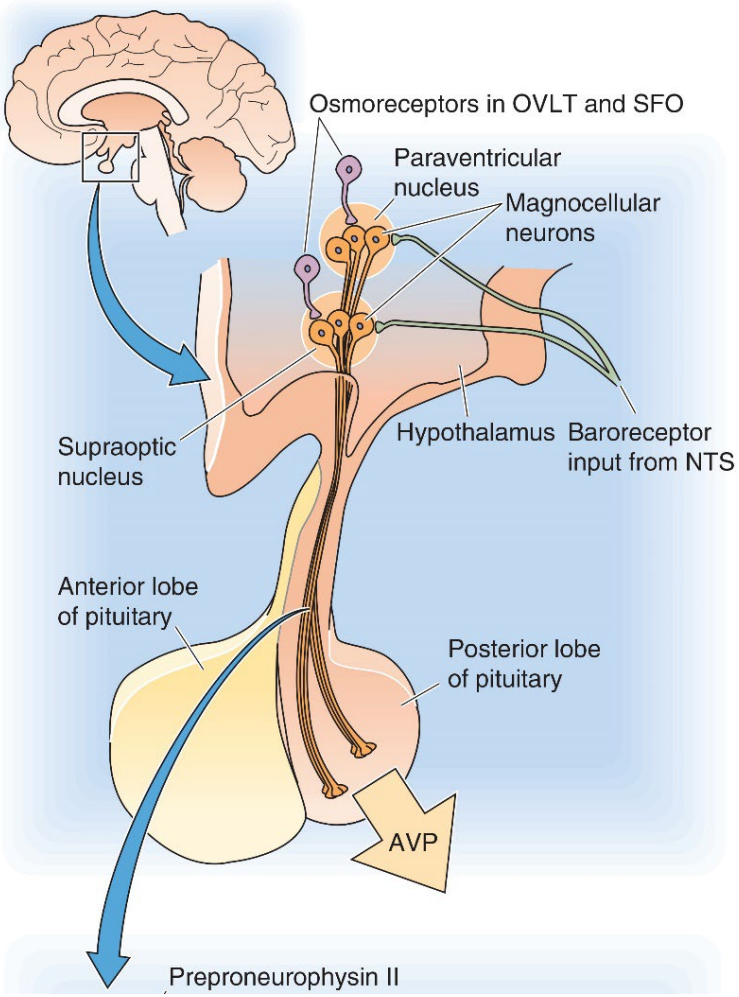
=> a humoral signal (hormone) causes the **decrease in urine production**

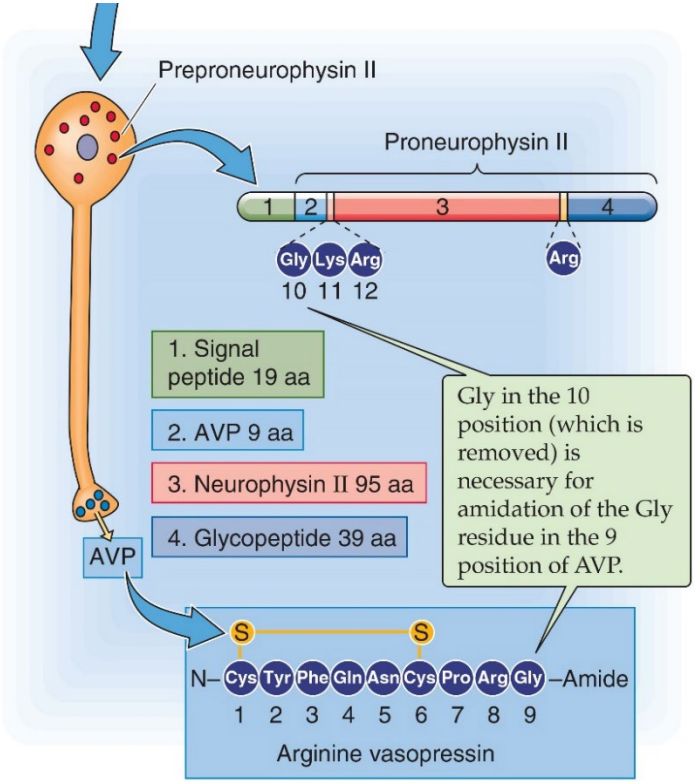
Boron Fig. 40-6

original experiment from Verney 1947, Proc. Royal Soc. London B

An increase in blood osmolality:

=> activation of osmo-sensitive neurons in **SFO** and **OVLT** (see below)
- these synapse onto **magnocellular neurons** in SON and PVN
→ → which release of Arginine-vasopression (AVP) from their axons in posterior pituitary into the bloodstream





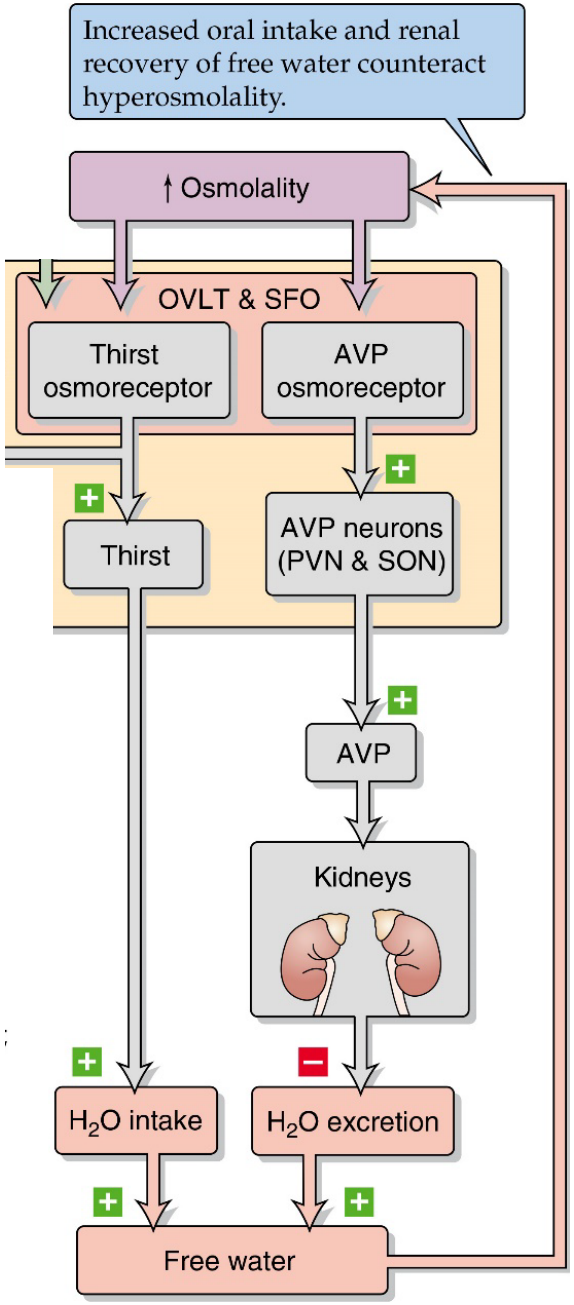
Boron Fig. 40-8

=> AVP (also called Antidiuretic hormone ADH):
↑ H_2O re-absorption in collecting duct of the kidney => less urine; more concentrated

b) Thirst (↔ oral intake of water)

Activation of the SFO and OVLT also lead to the "feeling of thirst"

=> increased water intake



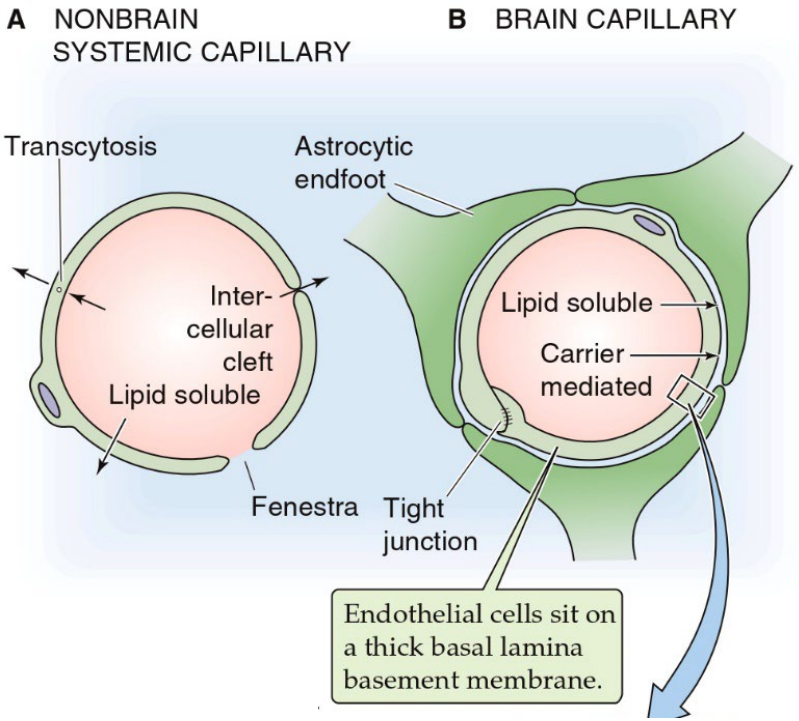
Boron Fig. 40-9

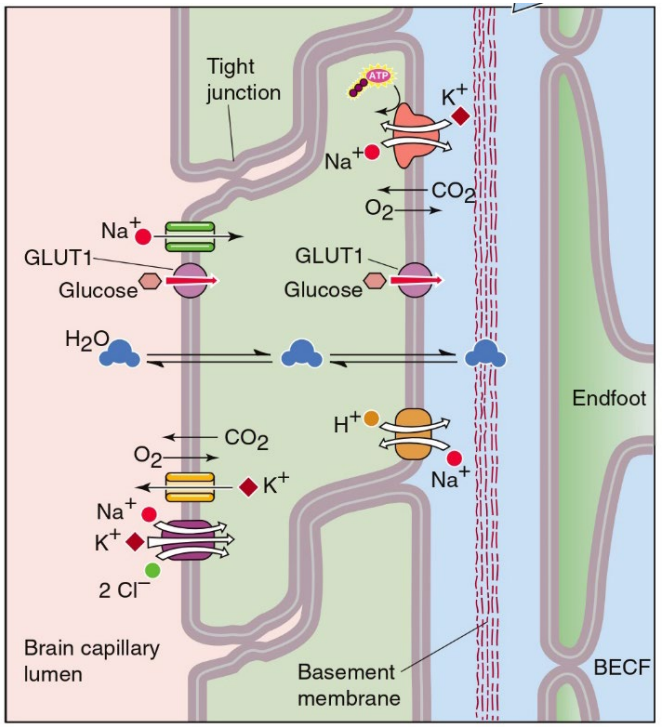
Circumventricular organs, SFO and OVLT

1) small detour: the blood-brain barrier (BBB)

The blood-brain barrier (BBB):

brain capillaries are tightened by endothelial cells + astroglia endfeet
(Brain capillaries are tighter than other capillaries in the body)

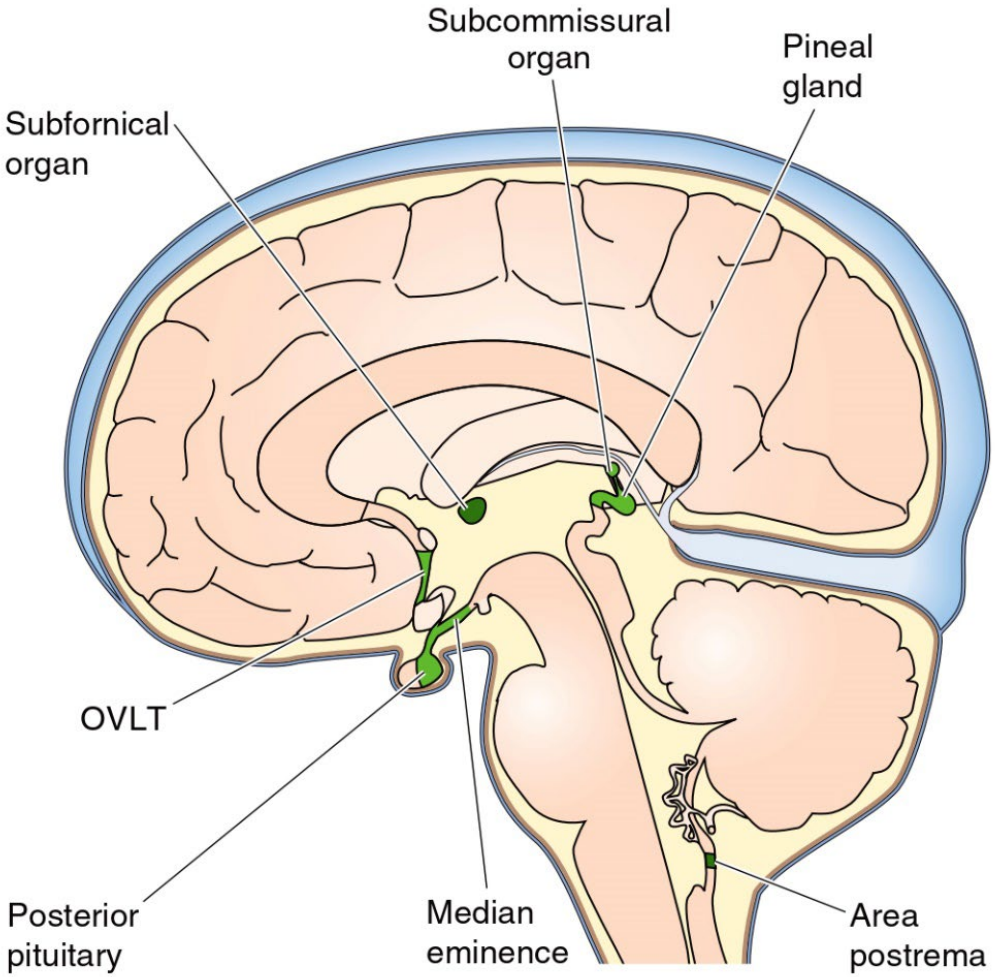




Permeable through BBB:
- H_2O (water channels)
- gases (O_2 , CO_2)
- lipophilic substances
(e.g. steroid hormones, estradiol, ect.)
- some drugs: EtOH, caffeine, nicotine, heroine

Not Permeable through BBB:
- ions
- proteins (esp. large ones)
- nutrients like glucose
need to be transported (GLUT1)

Now, at several areas, the brain has a "leaky" BBB



These areas are "circumventricular organs"

Relevant for **thirst**:

Subfornical organ, **SFO**

Organum vasculosum laminae terminalis (**OVLT**)

Boron Fig. 16-7

Vascularization of the rat OVLT

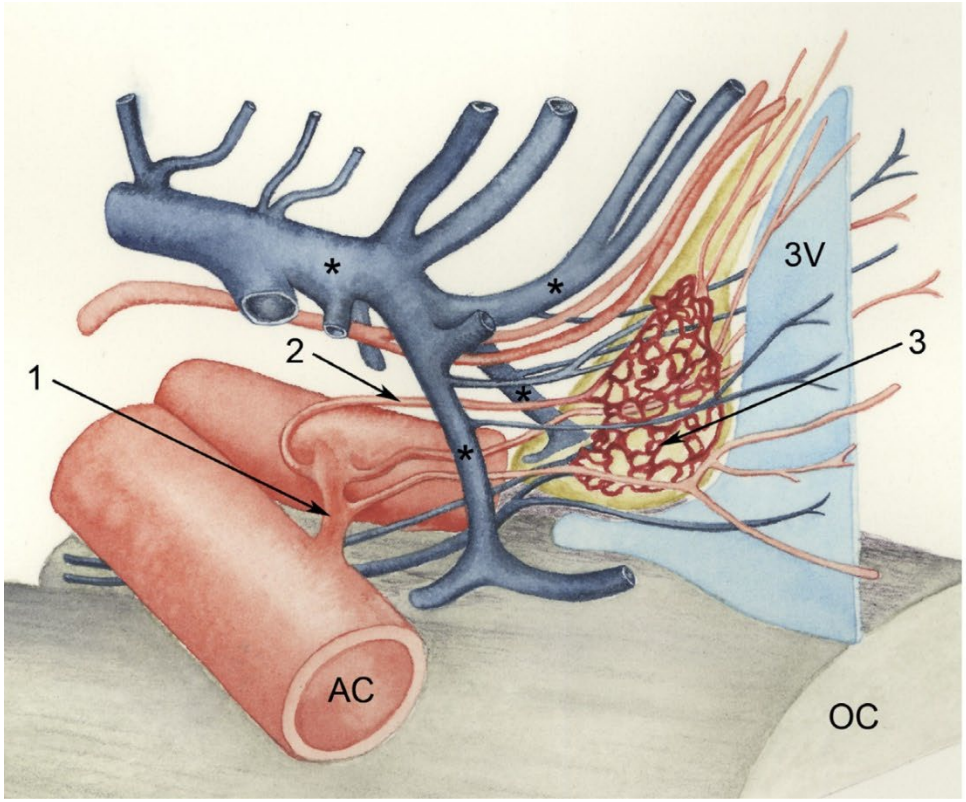
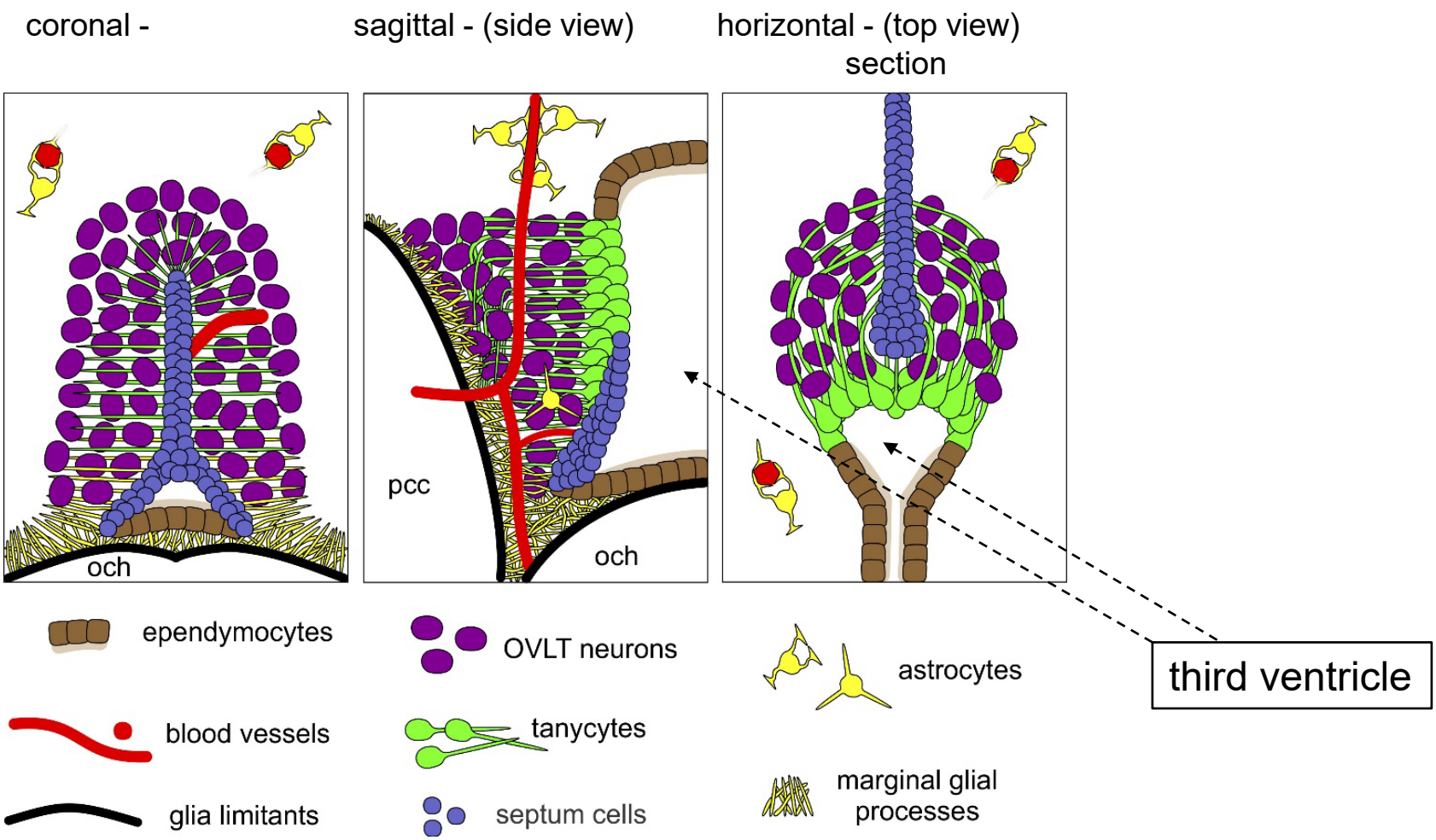


FIGURE 4 Side-view diagram of the vasculature supplying and draining the capillary plexus of the rat OVLT. The anterior communicating artery (1) has one or two branches (2) that supply the capillary plexus (3) of the OVLT. Venules coming from the OVLT drain into four larger veins (asterisked) surrounding the OVLT. *Other abbreviations*: AC, anterior cerebral artery, OC, optic chiasma; 3V, third ventricle. Based on the drawings and descriptions of Grafe and Weindl, 1987 and Mergner, 1961.

=> The circumventricular organs are the most vascularized structures in the brain

Oldfield & McKinley 2015, in "Rat Nervous System"

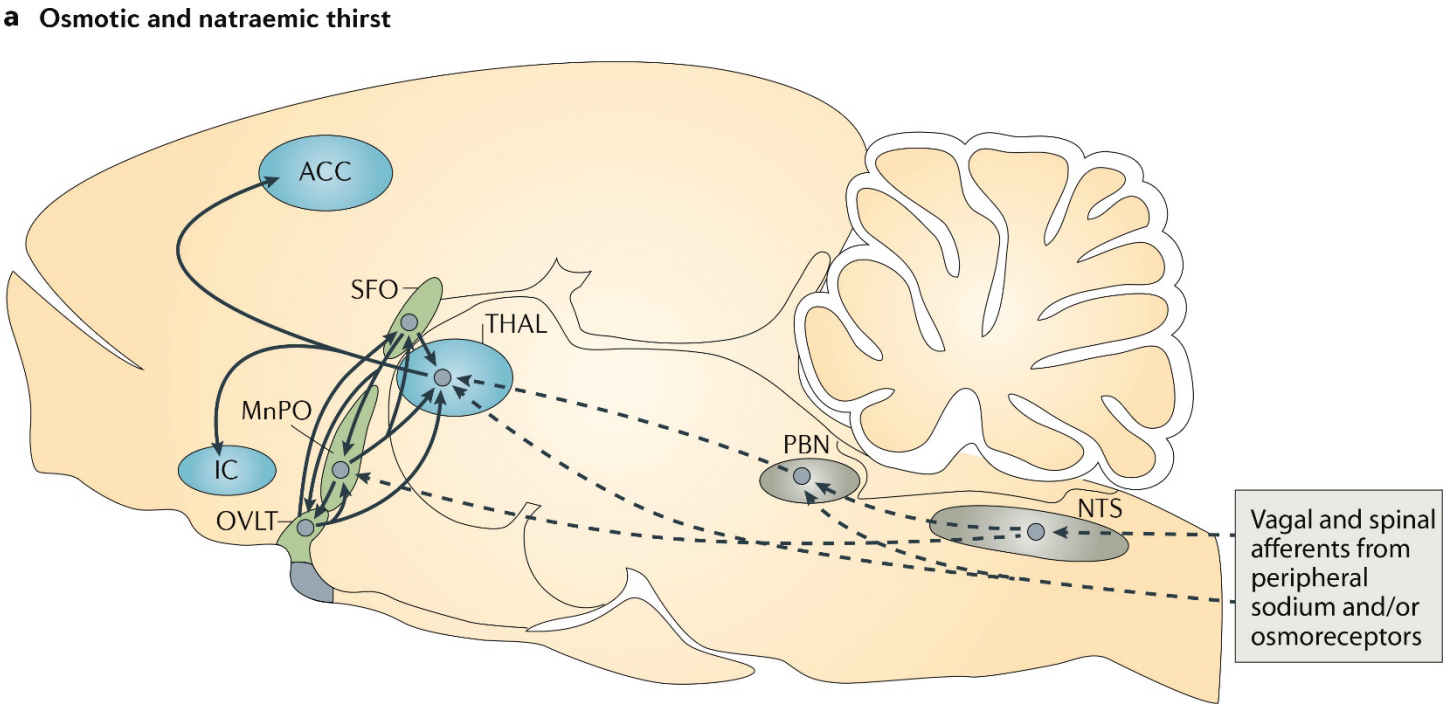
Anatomy and cell types in the OVLT



- tanycytes: tighten the OVLT neurons against the third ventricle
- also note: blood vessels without astrocytic endfeet in OVLT

Prager-Khoutorsky & Bourque 2015

Neuronal circuits involved in fluid regulation and thirst (overview)
1) **Osmotic insult** (hyperosmotic)

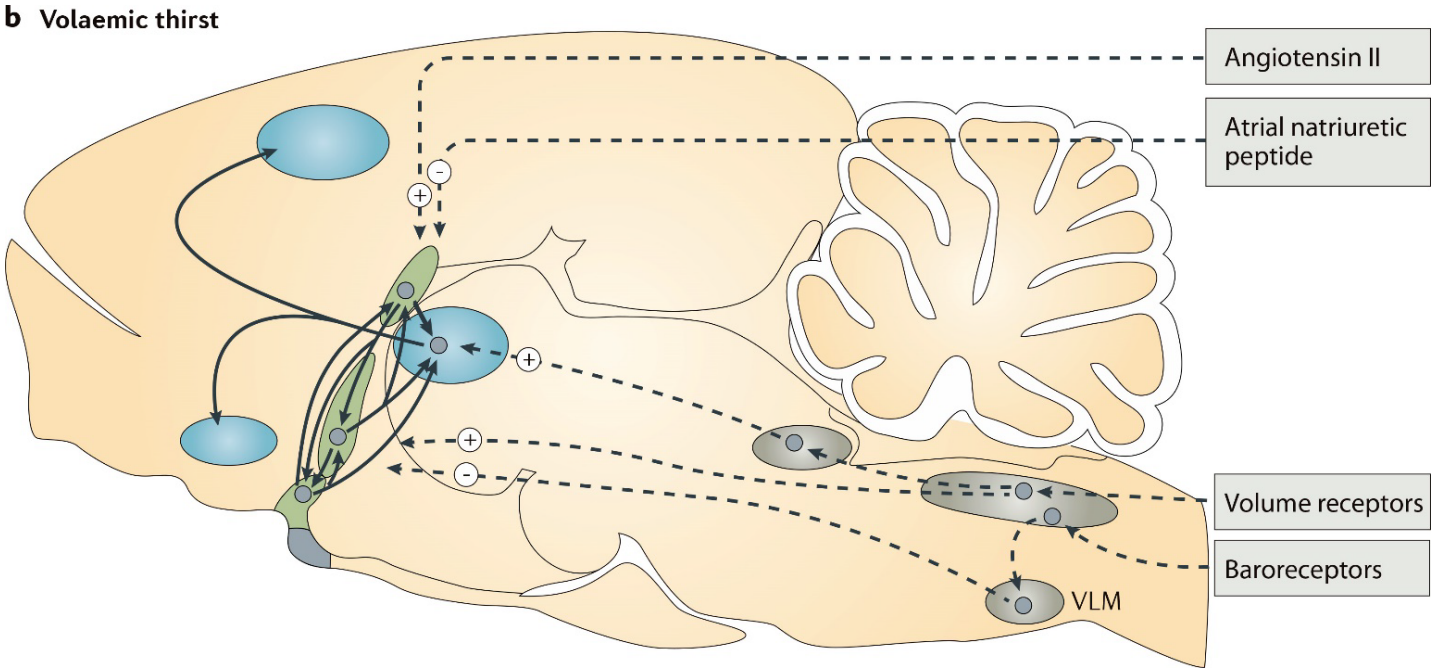


Gizoski & Bourque 2018, Nat. Reviews Nephrology

- Neurons in the circumventricular organs **SFO** and **OVLT** detect high blood osmolality + get depolarized, AP-firing
- They activate neurons in the MPO (medial preoptic nucleus)
- SFO, OVLT and MPO cause "thirst" drive

(probably via projections to midline thalamic nuclei (THAL), and from there to anterior cingulate cortex (ACC), and insular cortex (INS)

Neuronal circuits involved in fluid regulation and thirst (overview)
2) Volemic insult: low-pressure baroreceptor input to the thalamus (via N. vagus → Nucl. tractus solitarius) also comes into play



- In addition, in a **volemic insult**, Angiotensin II in blood is detected by receptors on SFO neurons and leads to their activation
(<=> see slide 22)

How does it work ?

LETTER

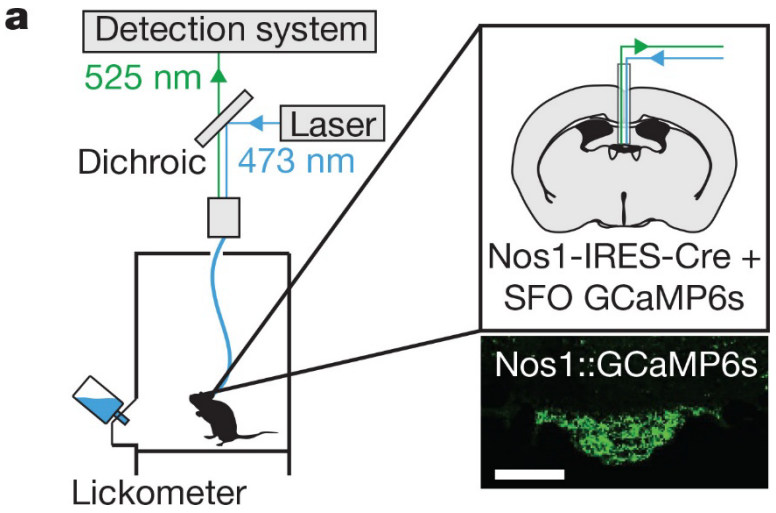
Nature, 2016

doi:10.1038/nature18950

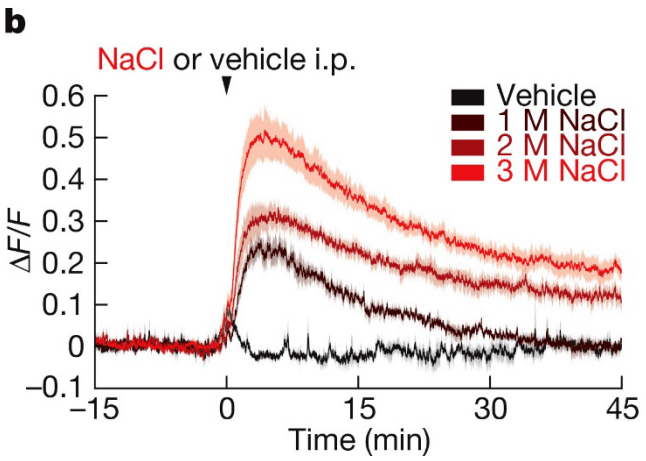
Thirst neurons anticipate the homeostatic consequences of eating and drinking

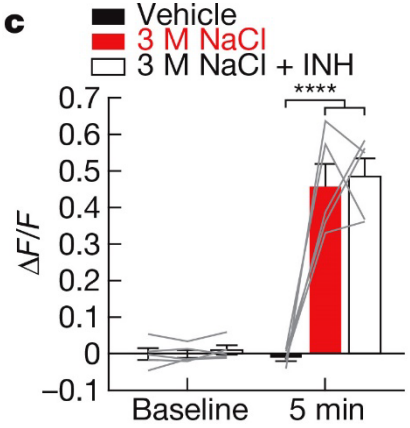
Christopher A. Zimmerman[1,2,3], Yen-Chu Lin[1,2], David E. Leib[1,2,3], Ling Guo[1,2,3], Erica L. Huey[1,2], Gwendolyn E. Daly[1,2], Yiming Chen[1,2,3] & Zachary A. Knight[1,2,3]

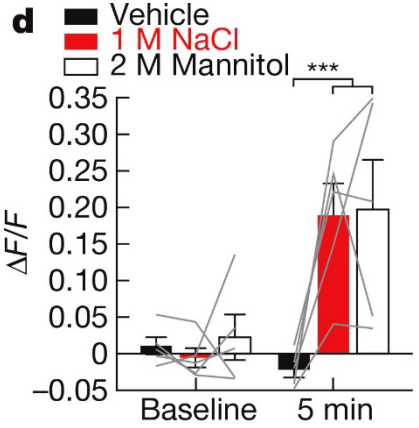
Fiber-photometry Ca^{2+} measurements (proxy of neural activity) in SFO NOS+ neurons during osmotic challenge



- <u>Osmotic challenge</u>:
Injection of of 1/2/3M NaCl solution intra-peritoneally



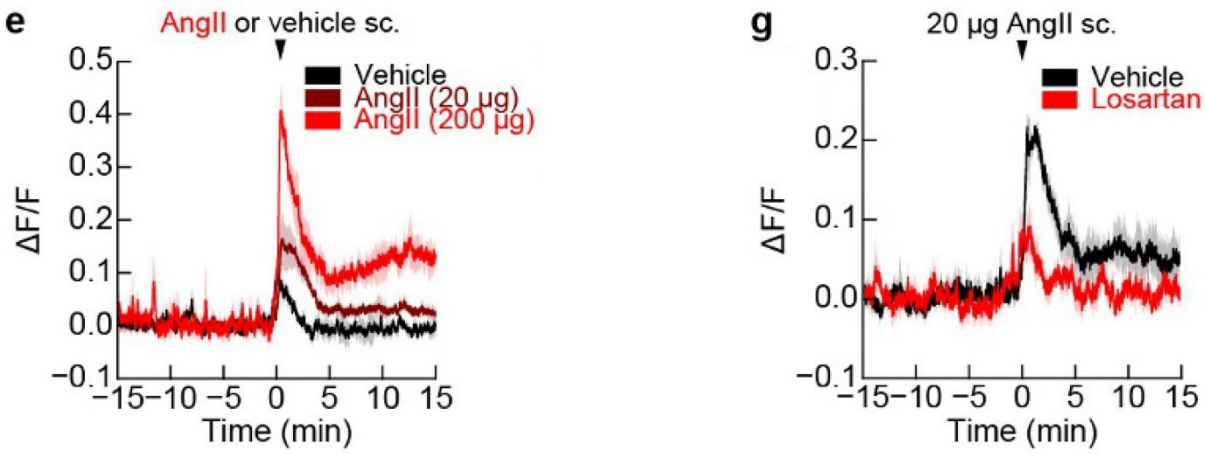




<u>note</u>: rise-time ~ 2 min, decay τ ~ 15 minutes

Zimmerman et al. 2016

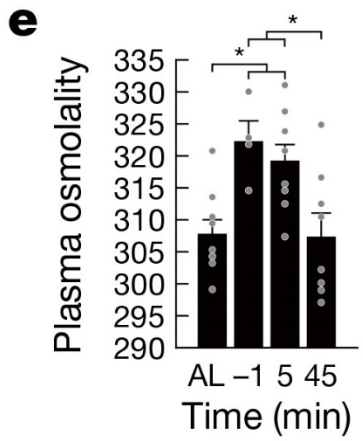
Angiotensin II injection also leads to an activation of SFO neurons (expected from classical findings)



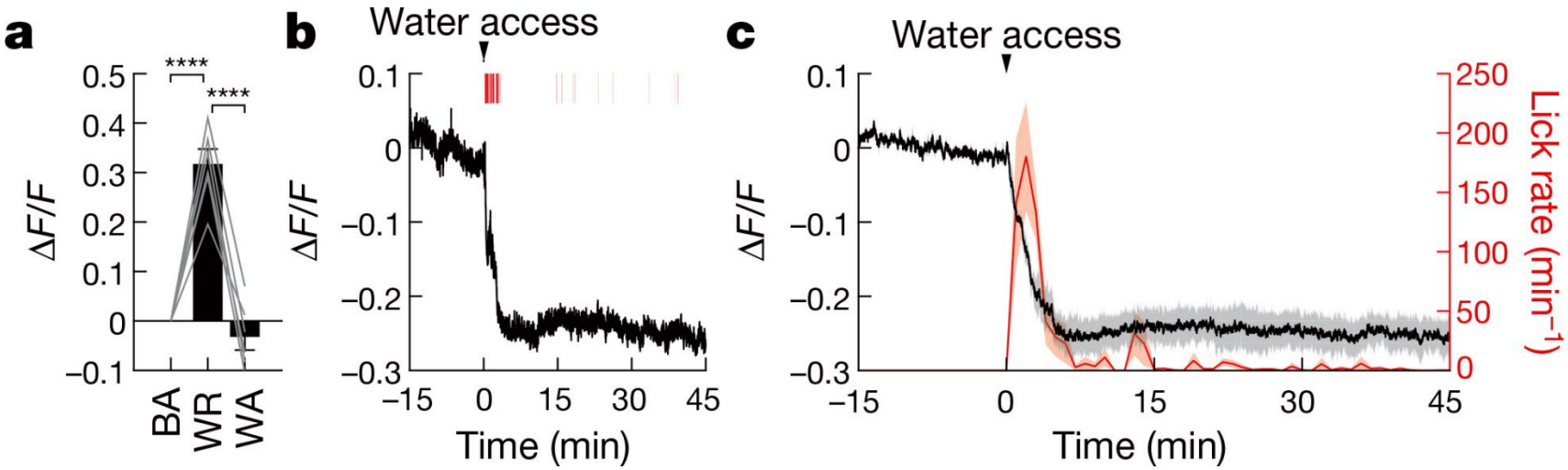
Physiological (normal) action of Angiotensin II in blood:
helps counteracting a hypovolemic insult via
- reducing Na^+ excretion from kidney (slide 9),
- causes thirst (slide 9 & 24)

Losartan: antagonist at the Angiotensin receptor (AT_1-R)

They then subjected mice to overnight water restriction, and measured blood osmolality, and Ca^{2+} in SFO neurons before & after renewed access to H_2O



e: Measured plasma osmolality during :
AL: ad libitum access (before H_2O restriction)
-1 : one minute before H_2O re-access
5 : five minutes after H_2O re-access & drinking
45: forty-five …



a: BA, baseline; WR: water restriction; WA: water access

Zimmerman et al. 2016

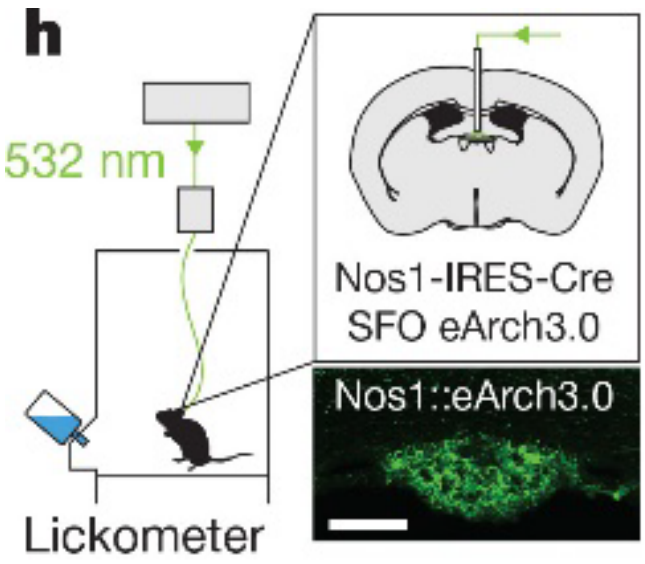
=> Activation of SFO neurons relaxes to normal within ~ 3 minutes of drinking,

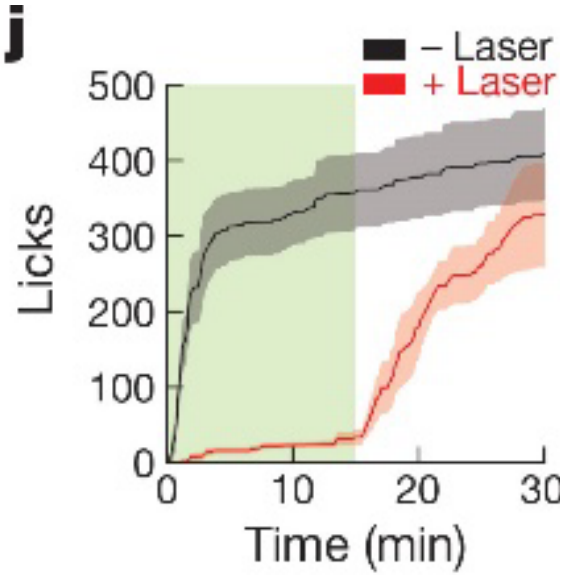
… much earlier than recovery of blood osmolality (the latter takes ≤ 45 min)

=> SFO "measures" the osmolality of blood ☑ (as known classically),
but SFO neuron's activity is also influenced by "anticipatory" signals.

=> Thirst is satiated earlier than re-establishment of blood osmolality

Optogenetic inhibition experiment: SFO neuron activity is necessary to drive licking (drinking) when H_2O restricted mice re-gain access to H_2O



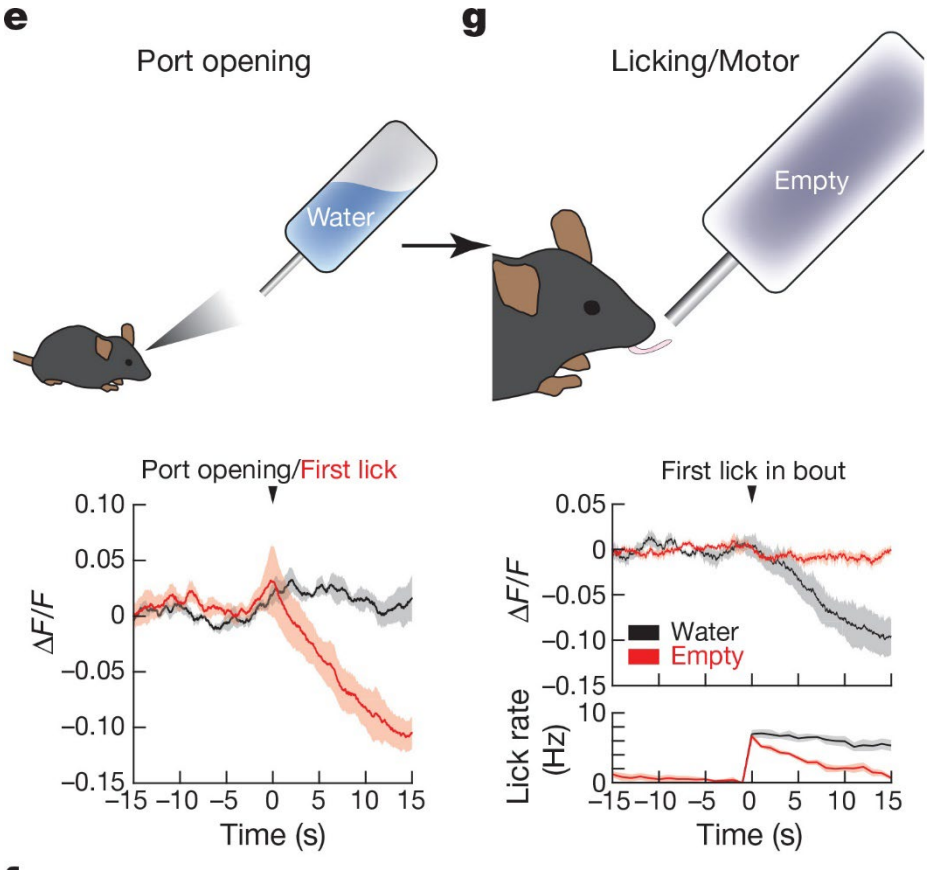


=> and also: *inhibition* of SFO activity is *sufficient* to stop drinking

Zimmerman et al. 2016

Further study of the nature of the anticipatory signals:

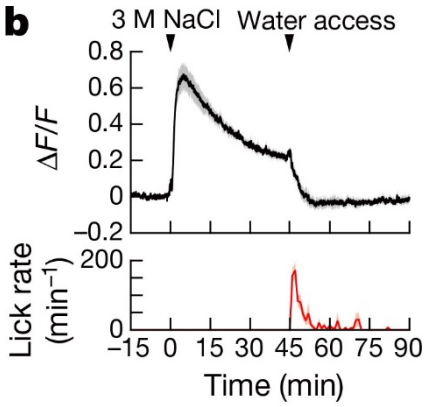
1) just seeing water, or licking on an empty bottle does not reduce SFO neuron activity:



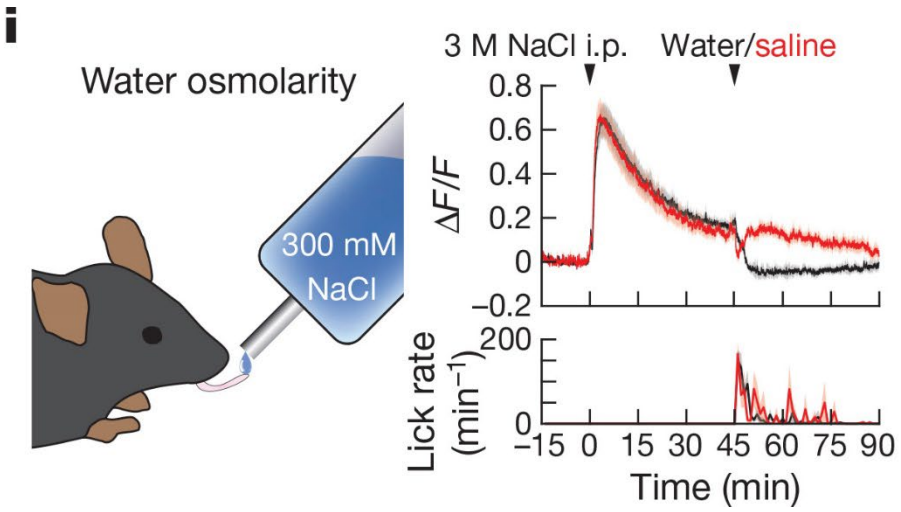
Zimmerman et al. 2016

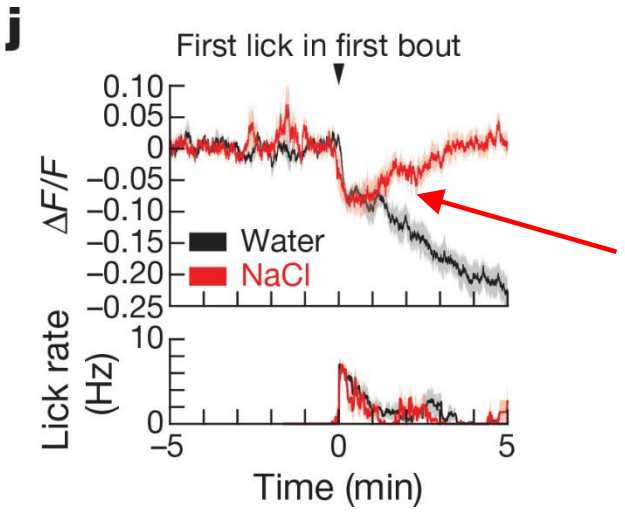
Further study of the nature of the anticipatory signals:

2) Drinking <u>hypertonic water</u> causes an initial, but not sustained, decrease in SFO activity:



b: Normal response to H_2O in mice
after hyperosmotic insult
(= licking, and fast & **complete** decrease of SFO activity)



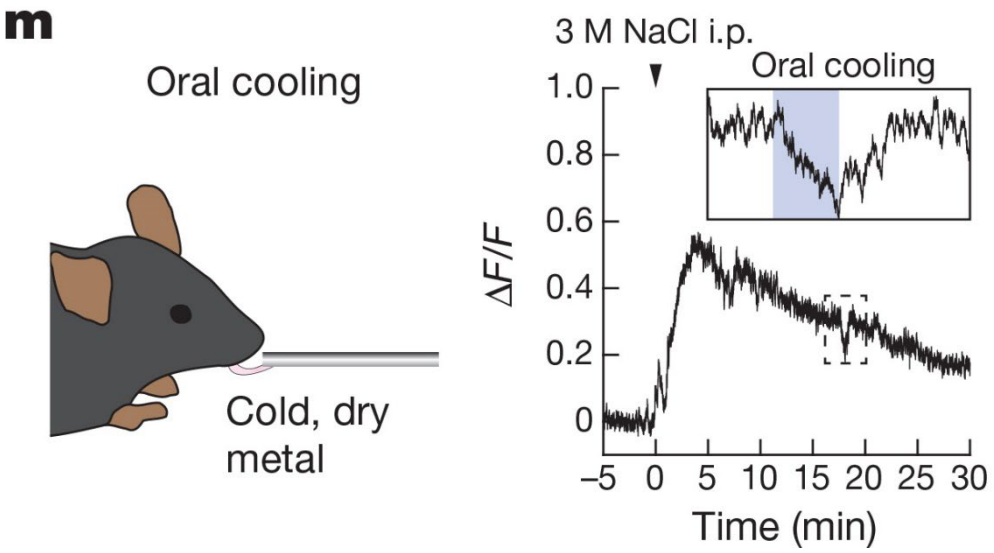


i, j: Hypertonic water!

Zimmerman et al. 2016

Further study of the nature of the anticipatory signals:

3) Licking on cold metal causes a small and transient reduction of SFO neuron activity



=> A signal from the oral cavity, especially cold liquid, is rapidly fed to the SFO

=> This rapid signal, together with more longer-lasting feedback on the recovery of blood osmolality, determines the switching-off of SFO neuron activity

Zimmerman et al. 2016

What is the neuronal mechanisms for the motivational drive to seek water in dehydrated state?

Is thirst "aversive" ?

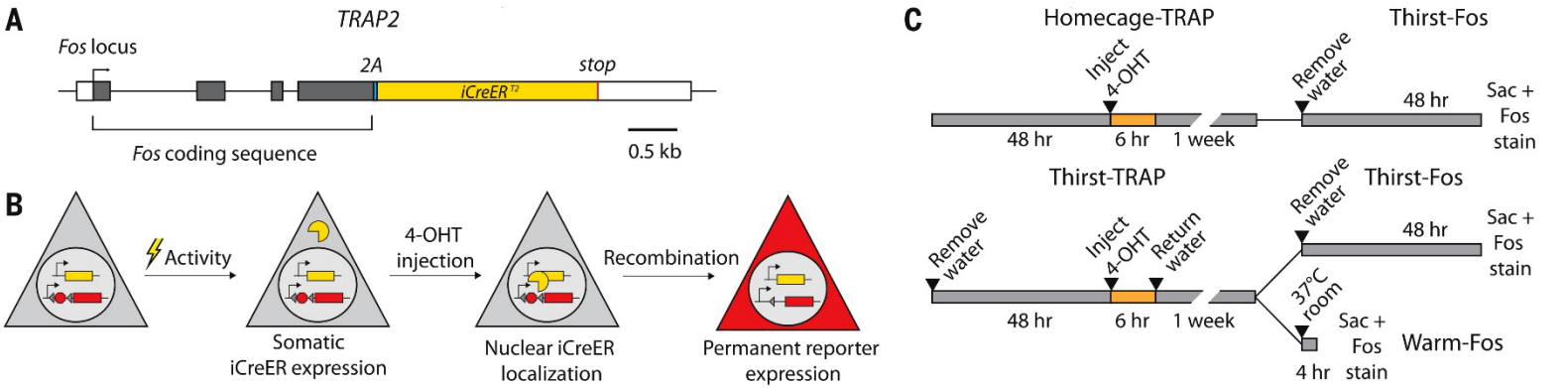
Science, 2017

NEUROSCIENCE

Thirst-associated preoptic neurons encode an aversive motivational drive

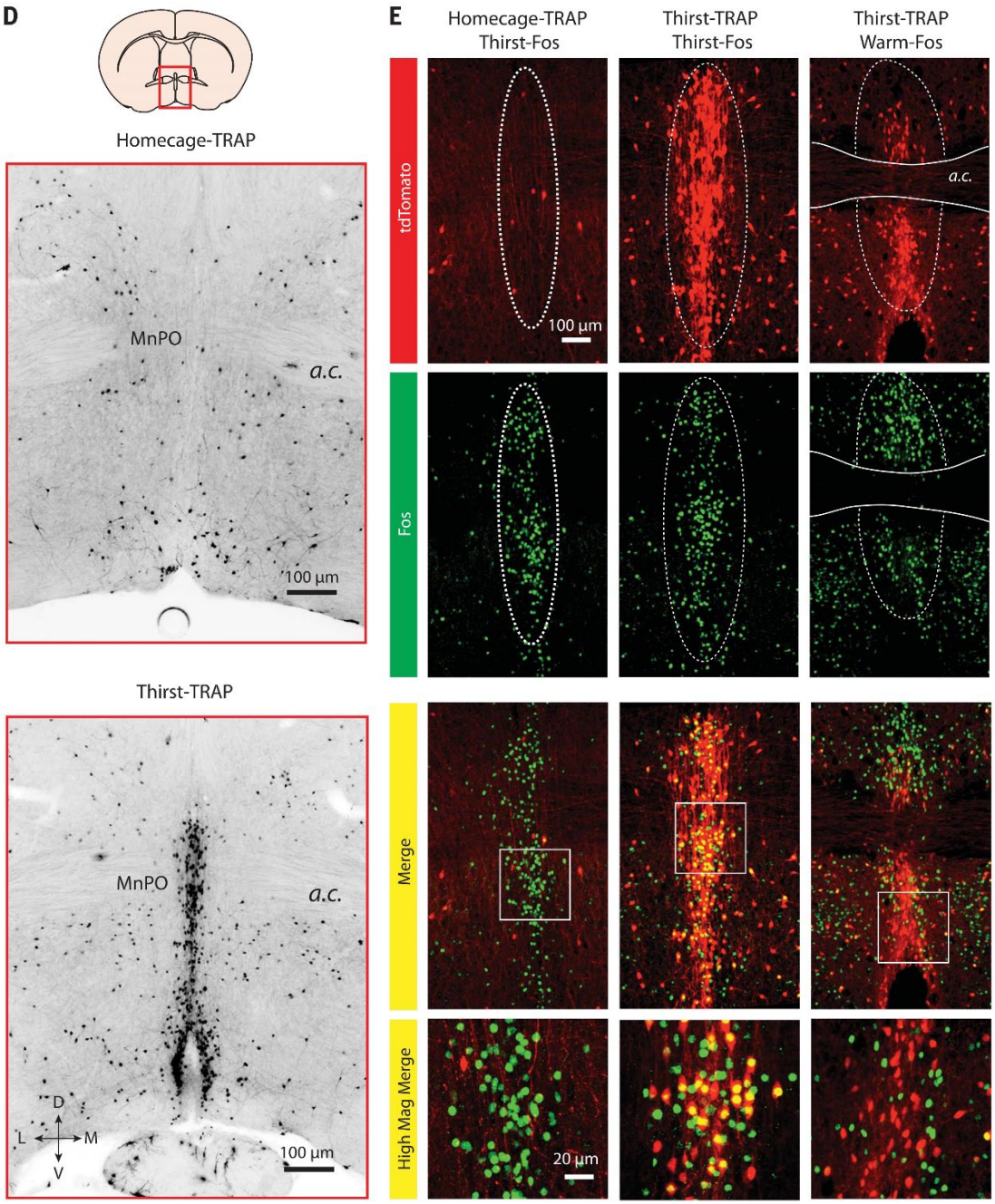
William E. Allen,[1,2]* Laura A. DeNardo,[1]* Michael Z. Chen,[1]* Cindy D. Liu,[1,3] Kyle M. Loh,[4] Lief E. Fenno,[6] Charu Ramakrishnan,[5] Karl Deisseroth,[3,5,6]† Liqun Luo[1,3]†

Method for activity-dependent genetic access to neuronal populations "TRAP2"



Allen et al. 2017

see also Guenthner et al. 2013
for the Method, from the same lab



Thirst-activated neurons can then be visualized by tdTomato-fluorescence

"Thirst-TRAPed" neurons are localized in the Medial preoptic nucleus (MnPO)

Homecage control does not "TRAP" many neurons (*later cFos-IHC to Thirst as a control*)

Warm stimulation activates a separate neuronal population

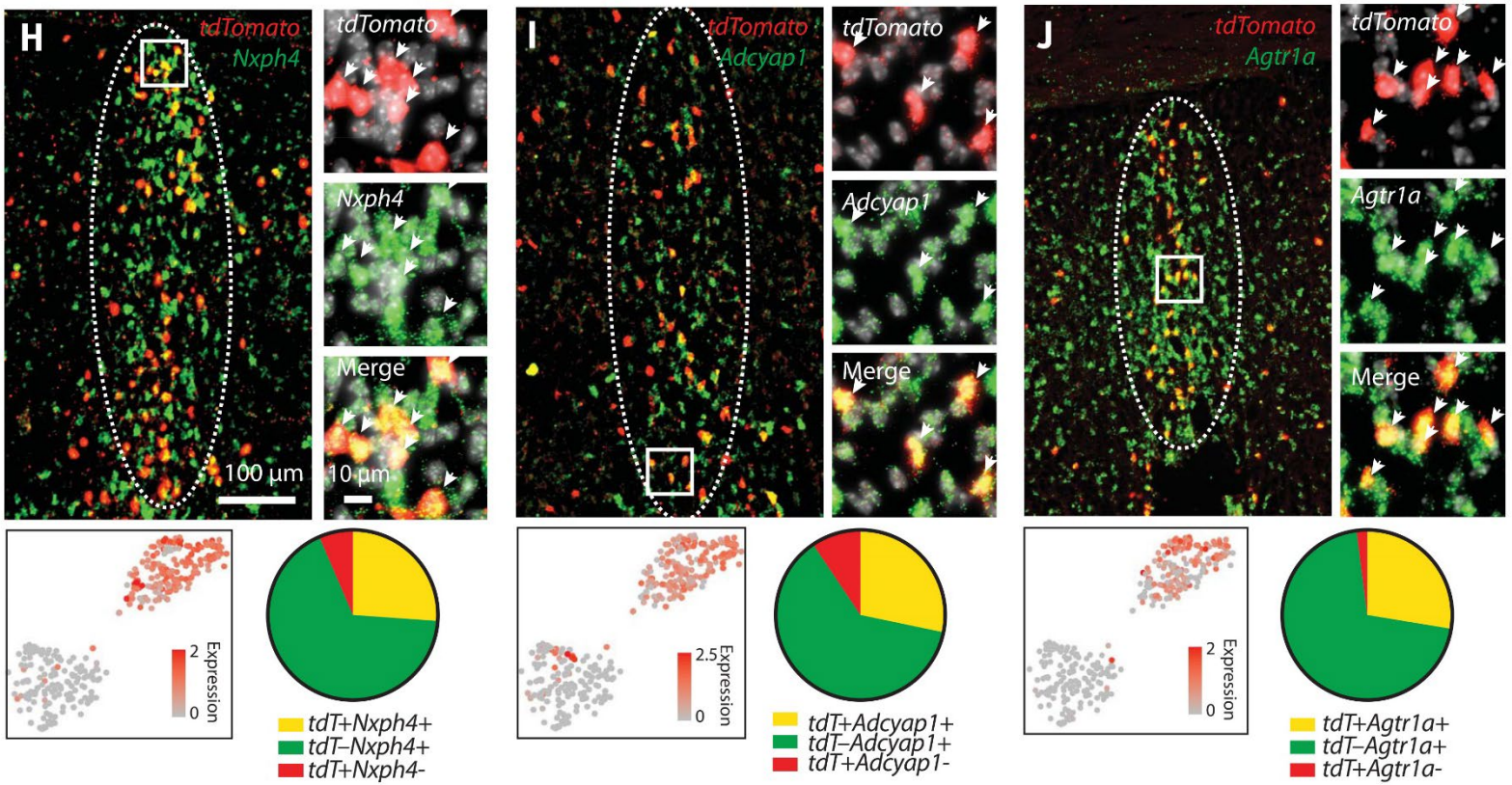
Allen et al. 2017

FACS sorting and sequencing of the Thirst-activated neurons in the MnPO showed two clusters of neurons:

1) A GAD1+ cluster (inhibitory GABA neurons, relatively heterogeneous)

2) A Slc17a6+ (VGluT2) cluster of excitatory neurons

- The excitatory cluster expresses several marker genes
- Their spatial expression was investigated by fluorescence in-situ hybridization (smFISH) in mice with "Thirst-TRAPed" MnPO neurons



=> Note: Agtr1a: Angiotensin II receptor; see above

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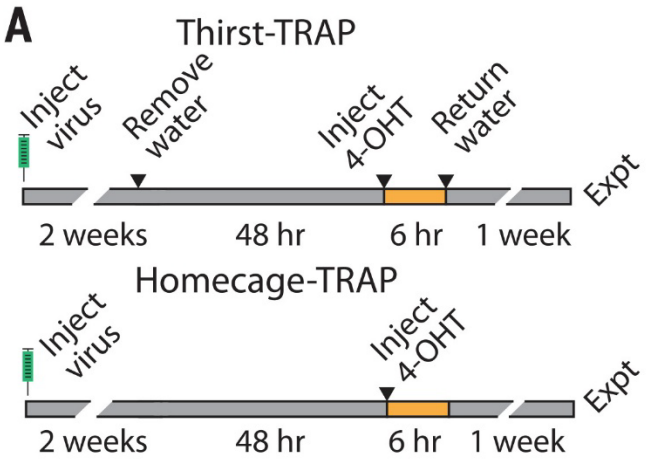
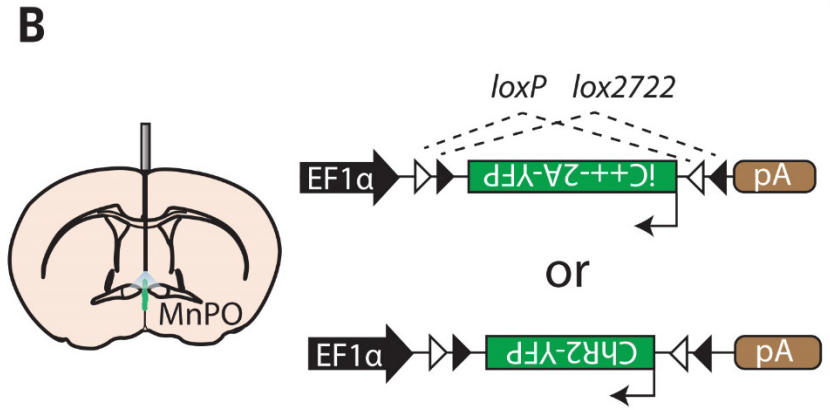
Next, they expressed channelrhodopsin-2 in a Cre-dependent manner in "Thirst-TRAPed" MnPO neurons





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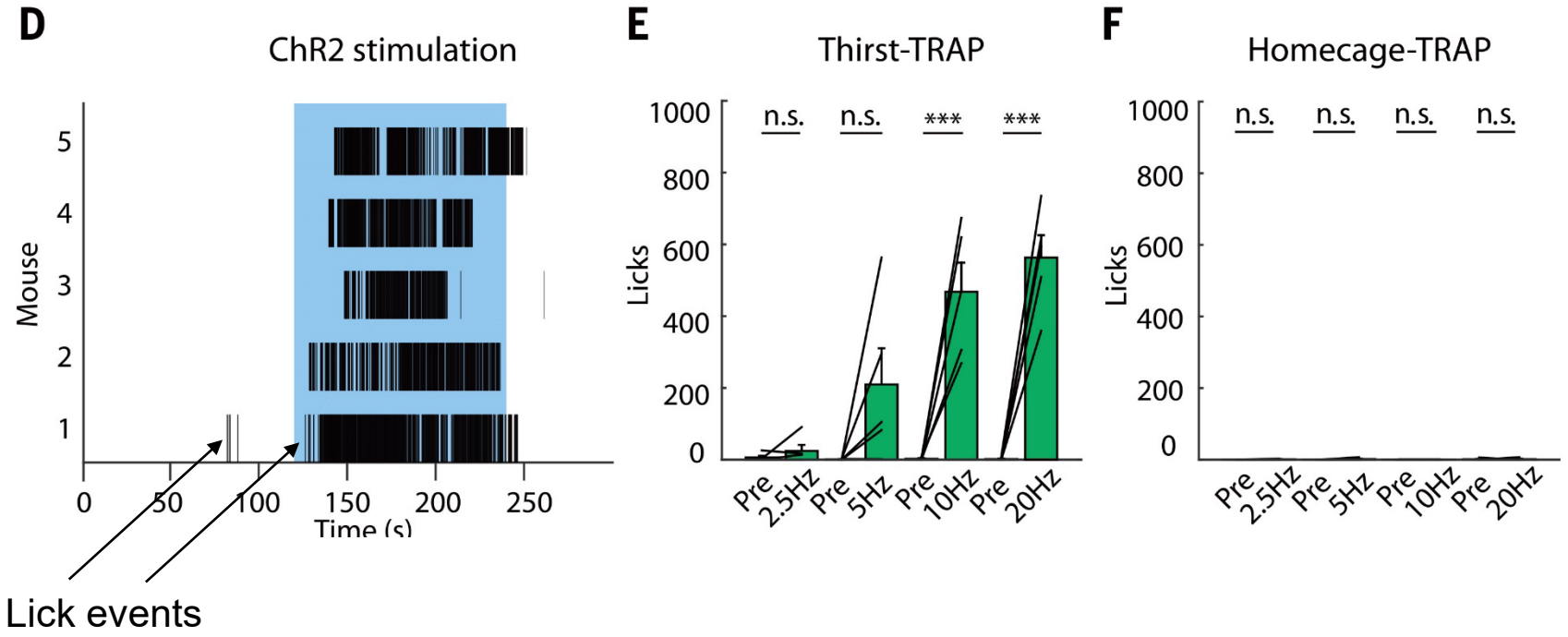
Optogenetic stimulation of Thirst-TRAPed MnPO neurons in water-sated mice leads to water consumption



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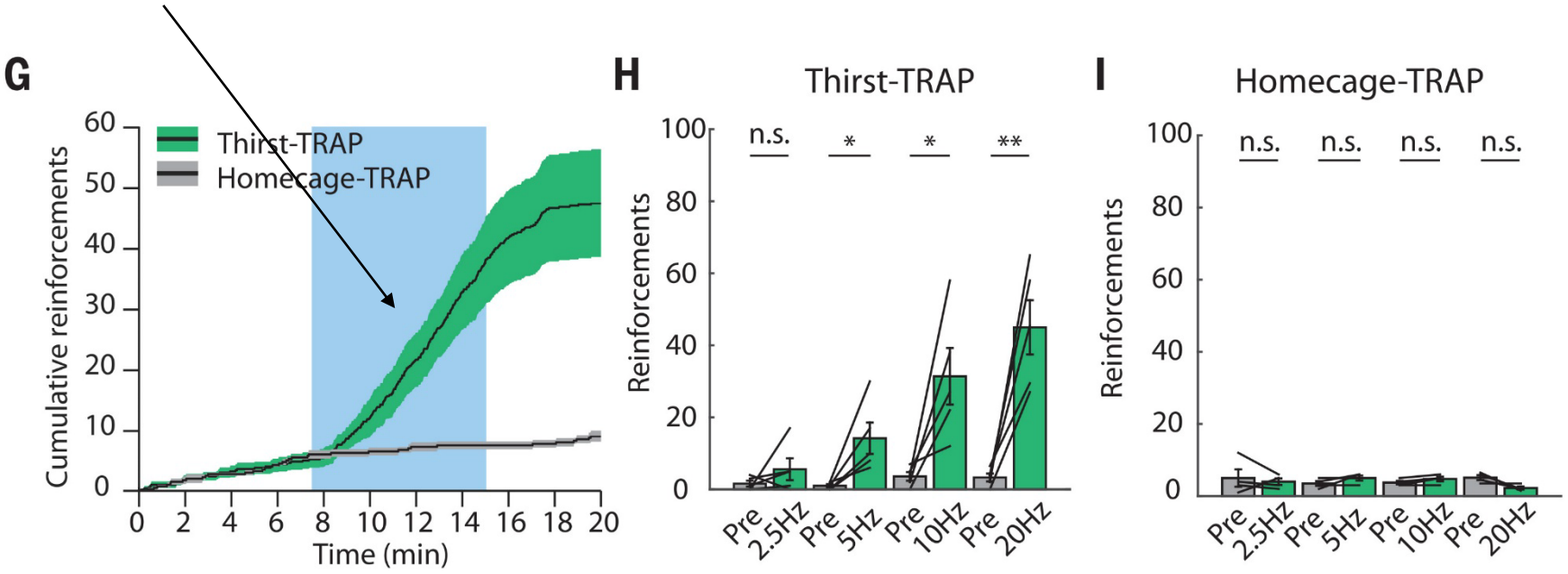
Does MnPO activation increase the drive of mice to "work" for water?

Experiment:
- Mice first learn to press a lever for water (operant learning task)
- Later, "Thirst-TRAPed" neurons are activated (ChR2) in water-satiated mice:
 This drives a dose-dependent ↑ in lever presses !



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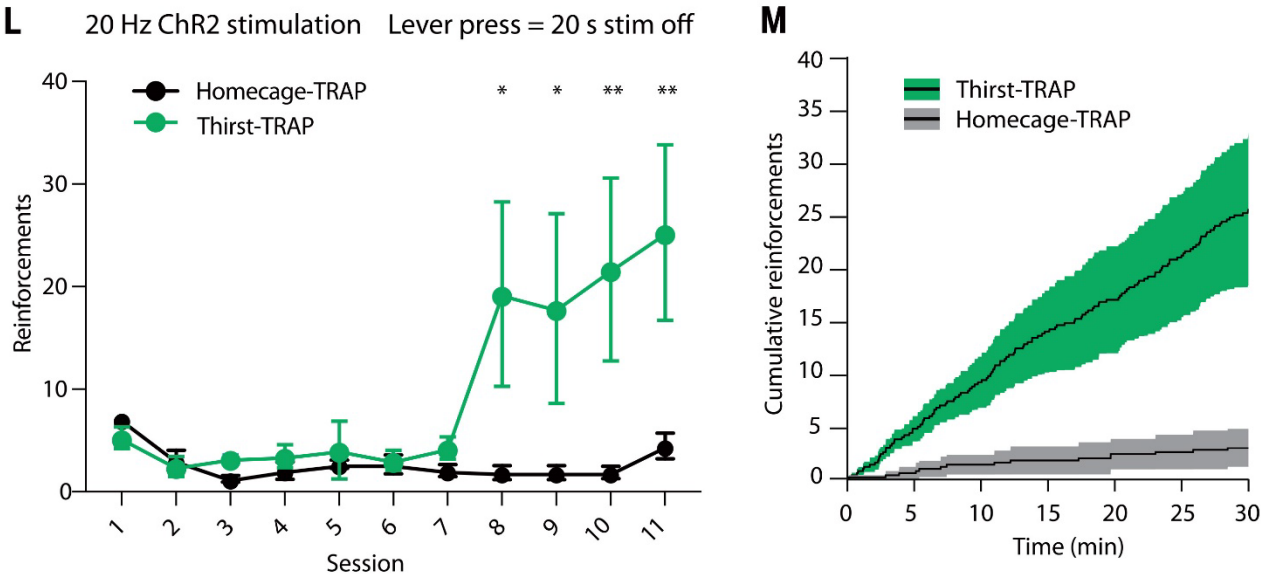
Lastly, mice learn to press a lever,
in order to **shut-off** optogenetic stimulation of MnPO "Thirst-TRAPed" neurons



=> Mice "work" to switch-off the activity of the these neurons
(see above, or more indirectly by lever-pressing for water, which then also reduced MnPO neuron activity - see 1 slide above)

=> Activity of specific, thirst-activated neurons in the MnPO is aversive for mice

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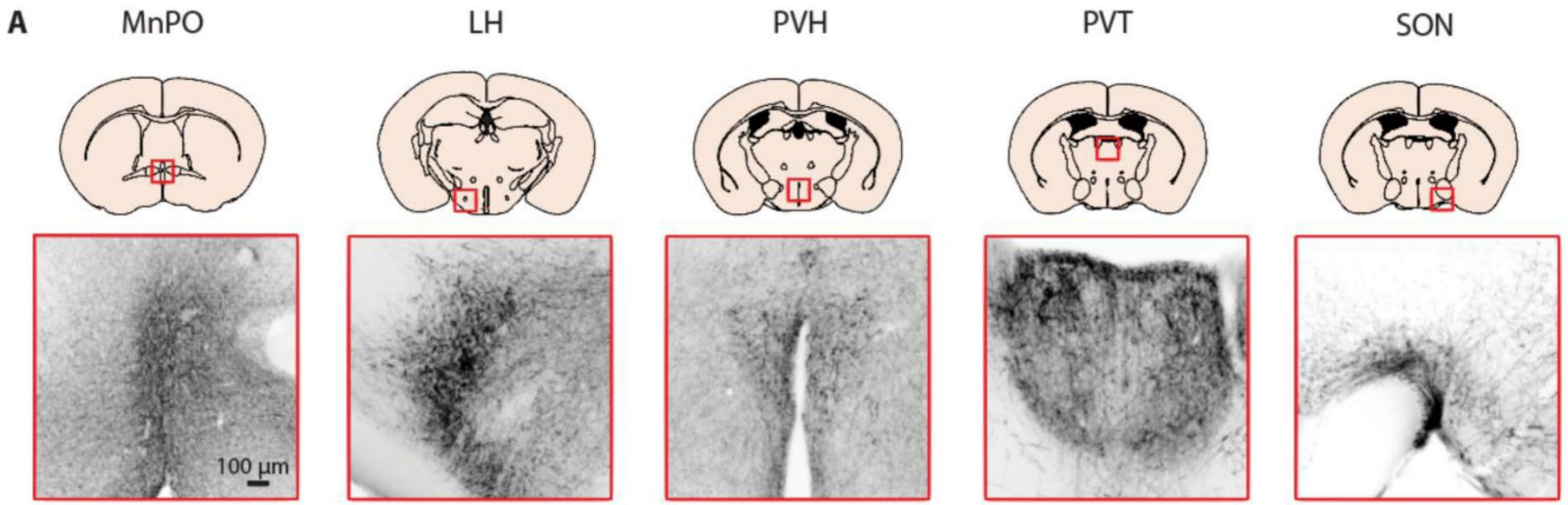
Thirst-TRAPed MnPO neurons project to various thalamic and hypothalamic downstream targets



(ChannelRhodopsin-2-YFP was expressed in a Cre-dependent manner in "Thirst-TRAPed" MnPO neurons as above)

(Section were stained for YFP)

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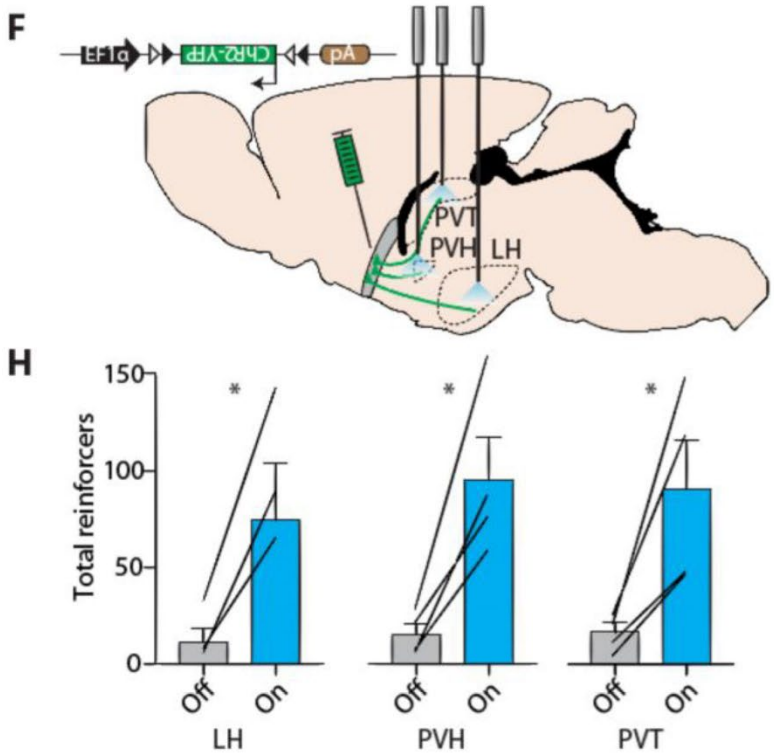
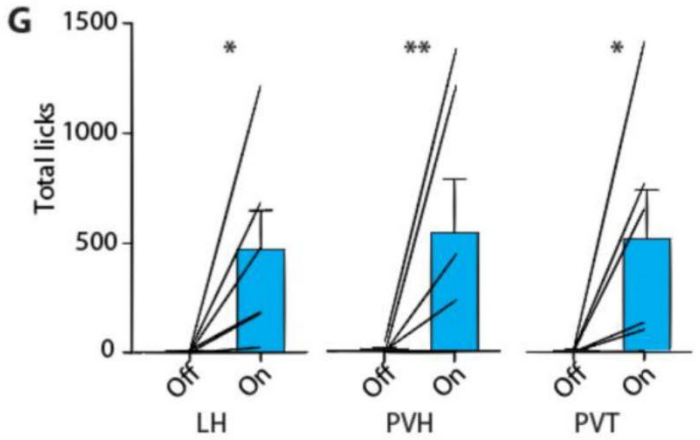
And: Optogenetic stimulation of the Thirst-TRAPed MnPO neurons in the LH, PVH and PVT stimulates water drinking (G) and lever-pressing for water (H)





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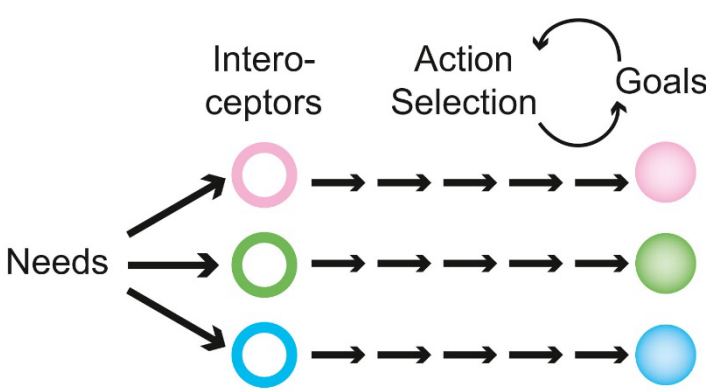
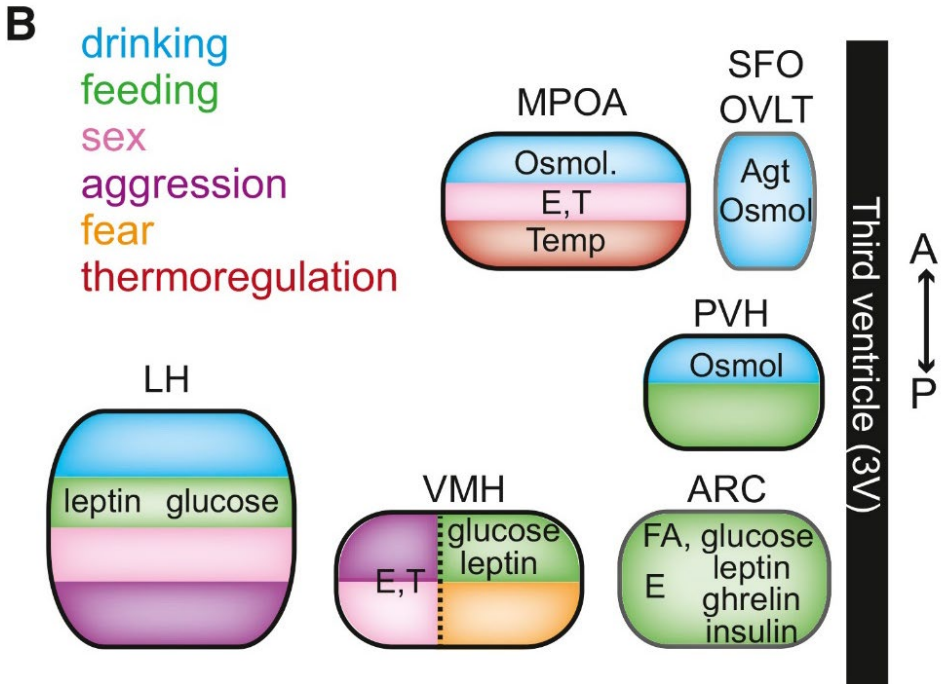
Overview of hypothalamic circuits and their involvement in various survival-related behaviors





Sternson 2013 Neuron, review

Paper reading for Monday **7.4.2025**:

Augustine, V., Gokce, S.K., Lee, S., Wang, B., Davidson, T.J., Reimann, F., Gribble, F., Deisseroth, K., Lois, C., and Oka, Y. (2018). Hierarchical neural architecture underlying thirst regulation.
Nature *555, 204-209*. 10.1038/nature25488.

=> The paper is short, please prepare for discussing all 5 main Figures

Literature cited (the most important ones):

Yellow marked: On Moodle

Boron, Medical Physiology, Chapter 40:
"Integration of Salt and Water balance" (Giebisch, Windhager & Aronson)

Gizowski, C., and Bourque, C.W. (2018). The neural basis of homeostatic and anticipatory thirst. Nat Rev Nephrol *14, 11-25.*

Sternson, S.M. (2013). Hypothalamic survival circuits: blueprints for purposive behaviors. Neuron *77, 810-824.*

Augustine, V., Gokce, S.K., Lee, S., Wang, B., Davidson, T.J., Reimann, F., Gribble, F., Deisseroth, K., Lois, C., and Oka, Y. (2018). Hierarchical neural architecture underlying thirst regulation. Nature *555, 204-209*. 10.1038/nature25488.
(**Reading for April 7th**)

Allen, W.E., DeNardo, L.A., Chen, M.Z., Liu, C.D., Loh, K.M., Fenno, L.E., Ramakrishnan, C., Deisseroth, K., and Luo, L. (2017). Thirst-associated preoptic neurons encode an aversive motivational drive. Science *357, 1149-1155.*

Zimmerman, C.A., Lin, Y.C., Leib, D.E., Guo, L., Huey, E.L., Daly, G.E., Chen, Y., and Knight, Z.A. (2016). Thirst neurons anticipate the homeostatic consequences of eating and drinking. Nature *537, 680-684.*