Mechanisms of anaesthesia – from molecular targets to neuronal networks

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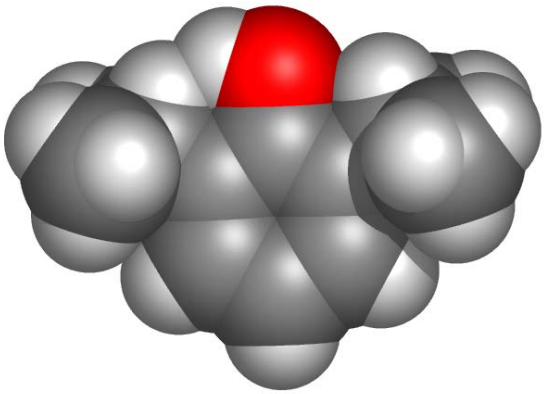
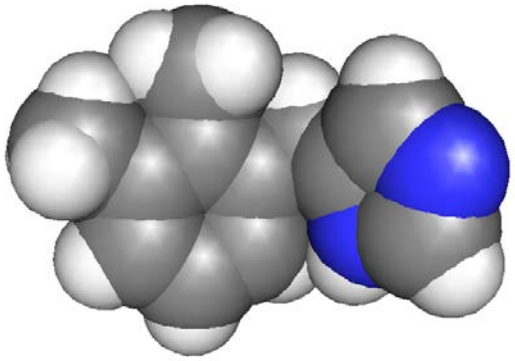


Propofol

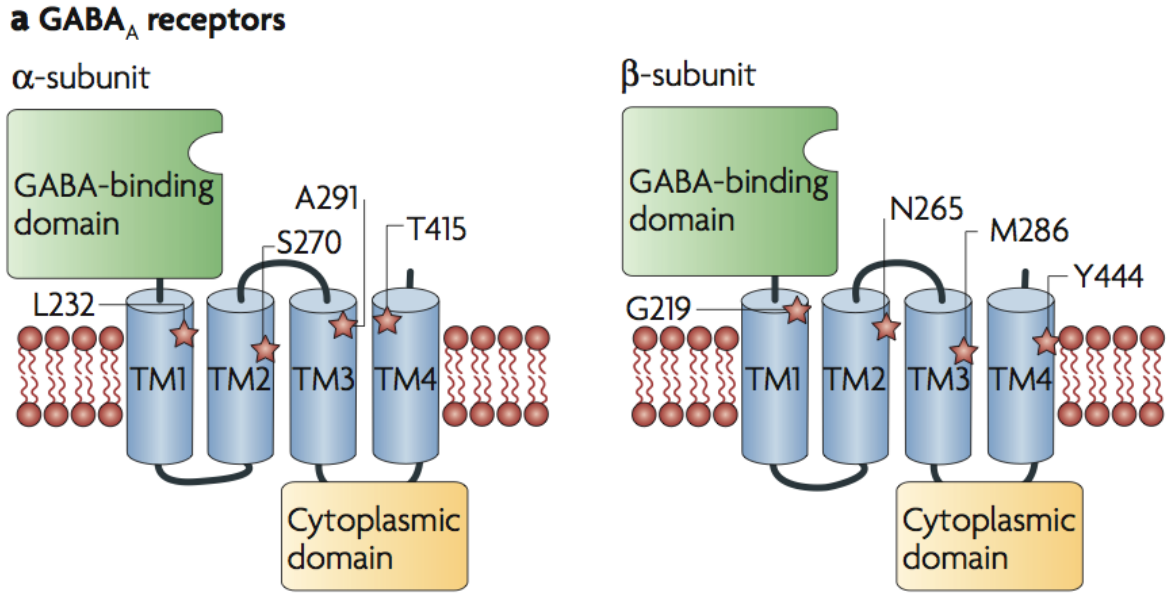
Potentiates inhibitory $GABA_A$ receptors

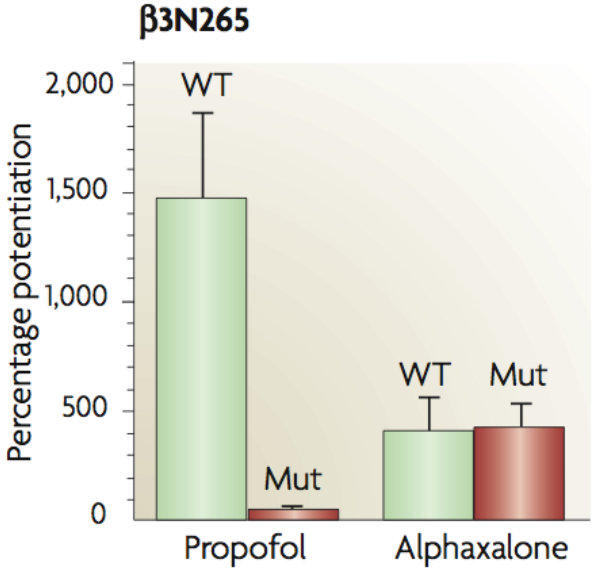


Dexmedetomidine

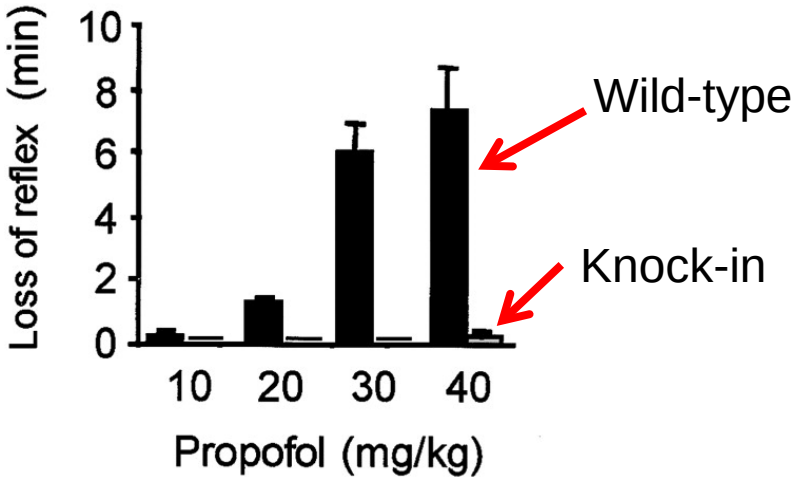
Activates inhibitory α_{2A} receptors

Knock-in mice can help determine the relevance of anaesthetic targets & pathways



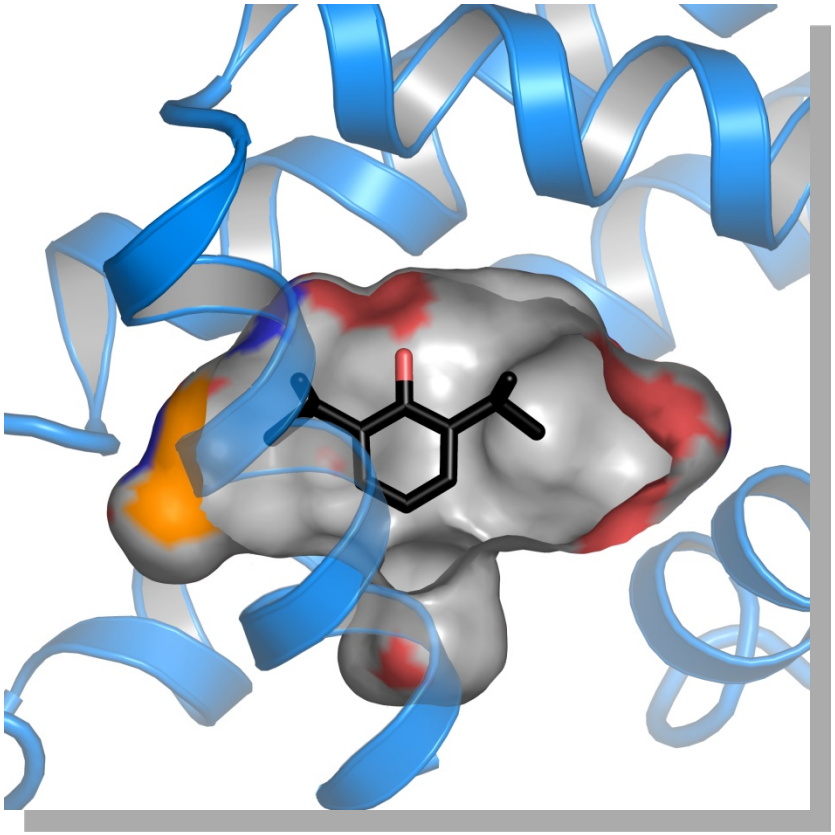


The β3N265M Knock-in mouse is insensitive to propofol

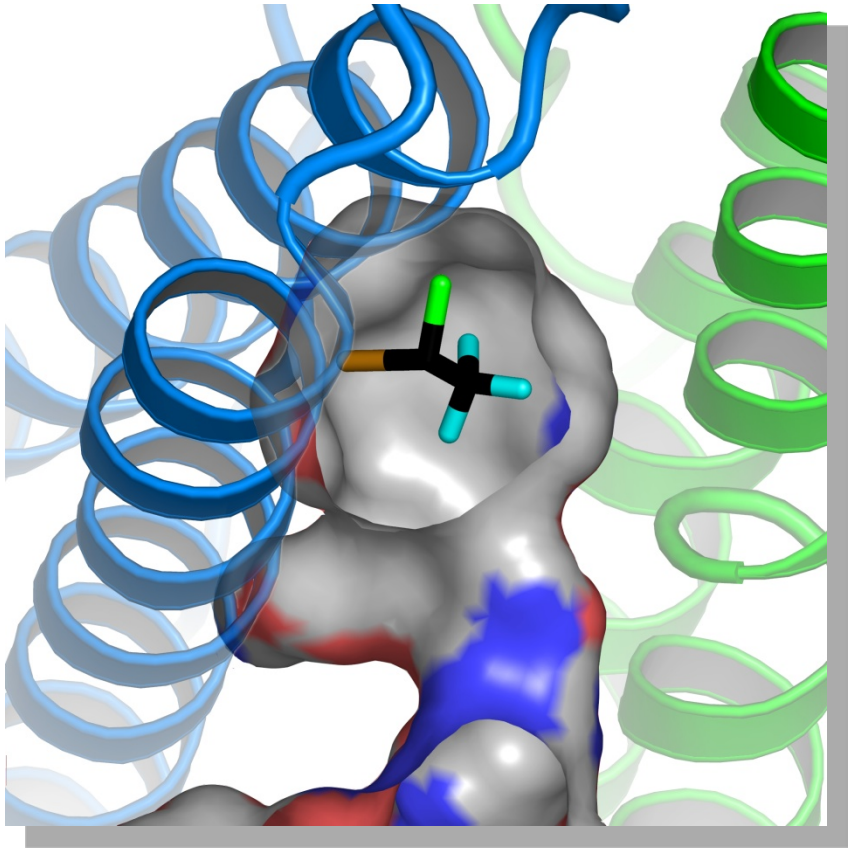


Jurd *et al. FASEB J.* **17,** 250 (2003)

Anaesthetics cause no structural changes at their binding sites



Bhattacharya et al. *JBC* **275,** 38731 (2000)



Liu et al. *FASEB J.*19, 567(2005)

nature chemical biology

ARTICLE

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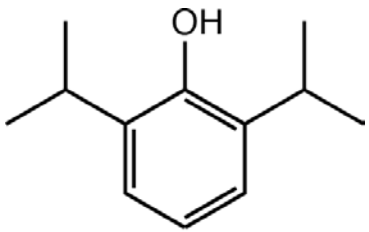
A propofol binding site on mammalian $GABA_A$ receptors identified by photolabeling

Grace M S Yip[1,7], Zi-Wei Chen[2,7], Christopher J Edge[1,3], Edward H Smith[1], Robert Dickinson[4], Erhard Hohenester[1], R Reid Townsend[5], Karoline Fuchs[6], Werner Sieghart[6], Alex S Evers[2,8]* & Nicholas P Franks[1,8]*

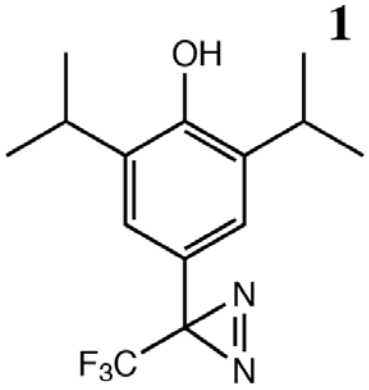
Propofol is the most important intravenous general anesthetic in current clinical use. It acts by potentiating $GABA_A$ (γ-aminobutyric acid type A) receptors, but where it binds to this receptor is not known and has been a matter of some debate. We synthesized a new propofol analog photolabeling reagent whose biological activity is very similar to that of propofol. We confirmed that this reagent labeled known propofol binding sites in human serum albumin that have been identified using X-ray crystallography. Using a combination of protiated and deuterated versions of the reagent to label mammalian receptors in intact membranes, we identified a new binding site for propofol in $GABA_A$ receptors consisting of both β_3 homopentamers and $\alpha_1\beta_3$ heteropentamers. The binding site is located within the β subunit at the interface between the transmembrane domains and the extracellular domain and lies close to known determinants of anesthetic sensitivity in the transmembrane segments TM1 and TM2.

Yip *et al. Nature Chemical Biology* **11,** 715 (2013)

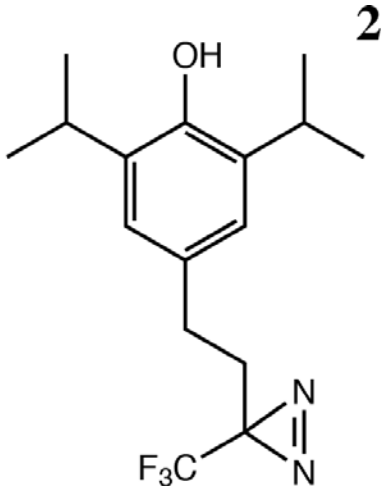
Photolabelling with *o*-propofol diazirine



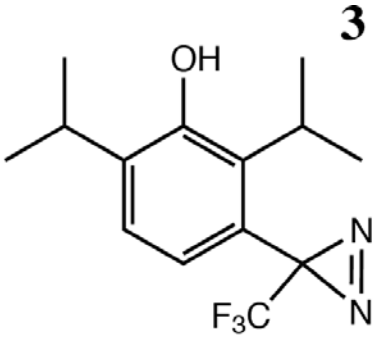
Propofol



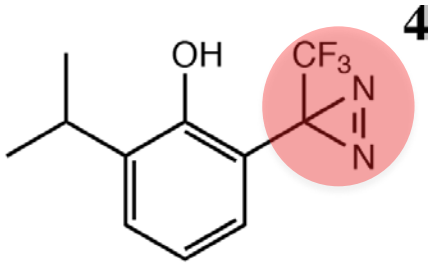
Para-propofol diazirine



Para-propofol dimethyl diazirine



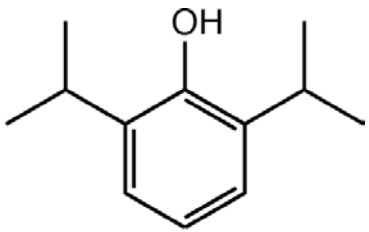
Meta-propofol diazirine



Ortho-propofol diazirine

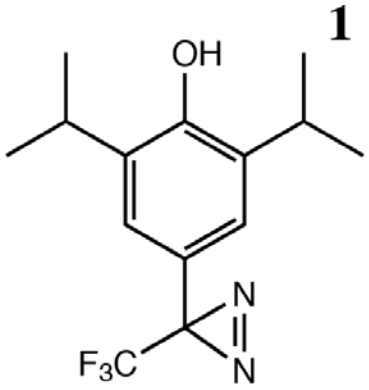
Yip *et al. Nature Chemical Biology* **11,** 715 (2013)

Photolabelling with *o*-propofol diazirine

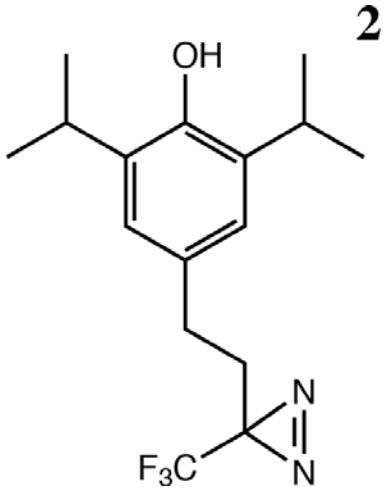


Propofol

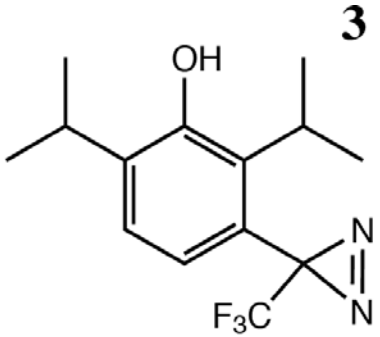
UV light >320nm



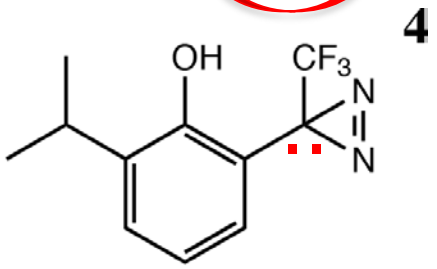
Para-propofol diazirine



Para-propofol dimethyl diazirine



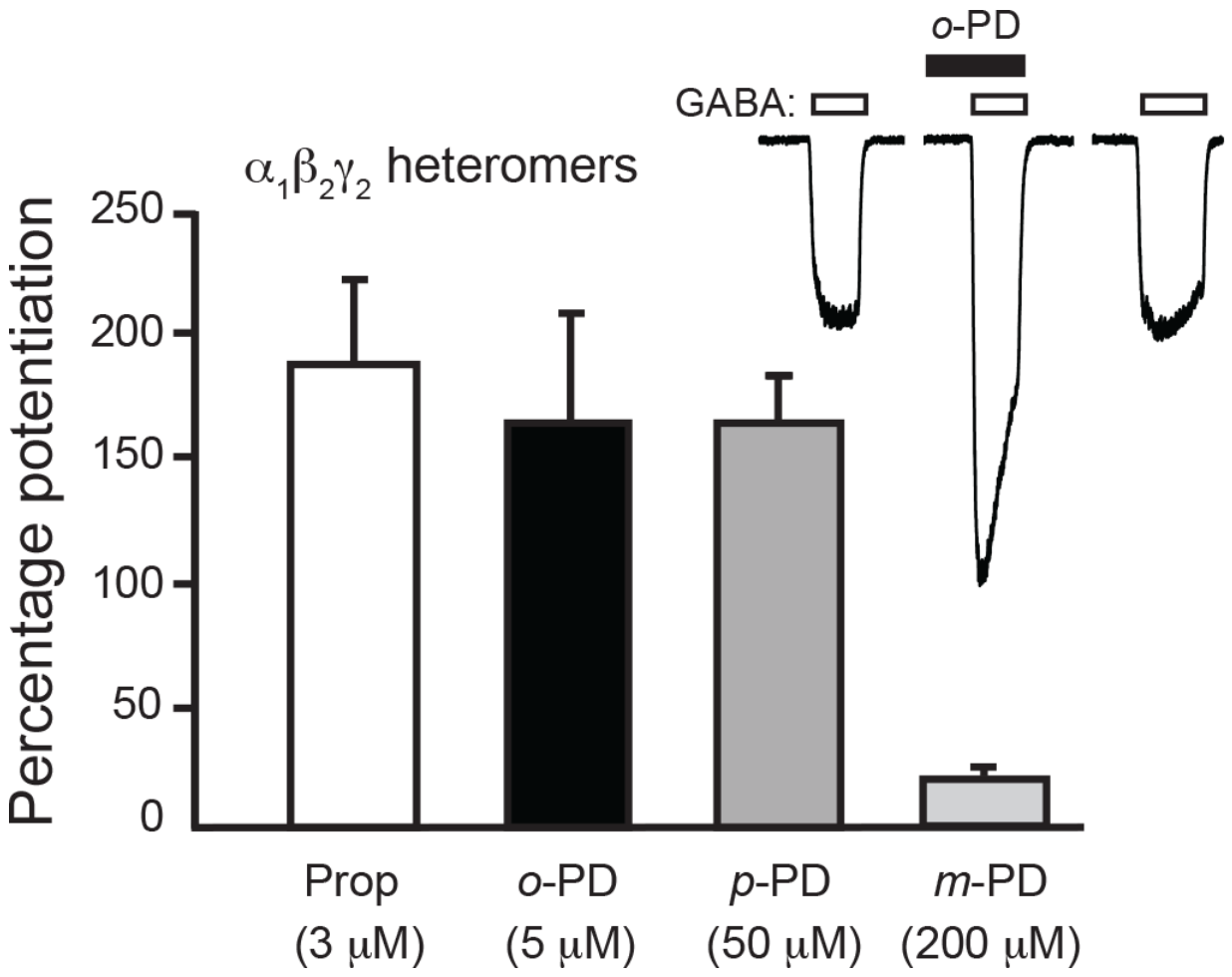
Meta-propofol diazirine



Ortho-propofol diazirine

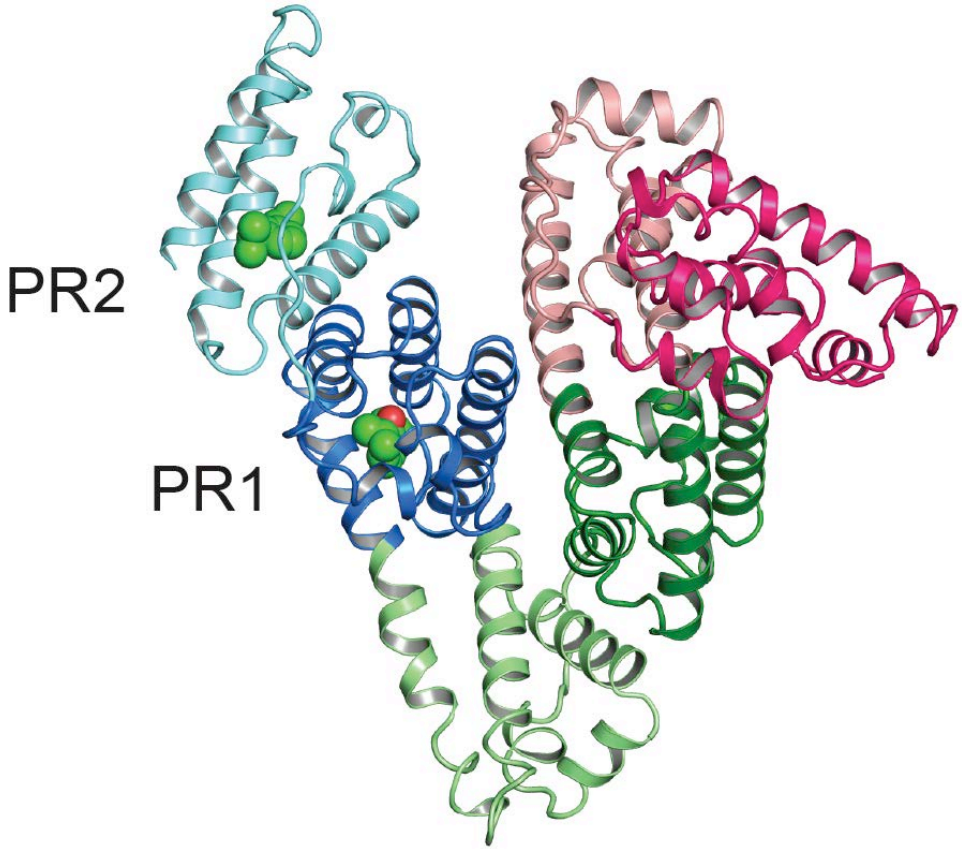
Yip *et al. Nature Chemical Biology* **11,** 715 (2013)

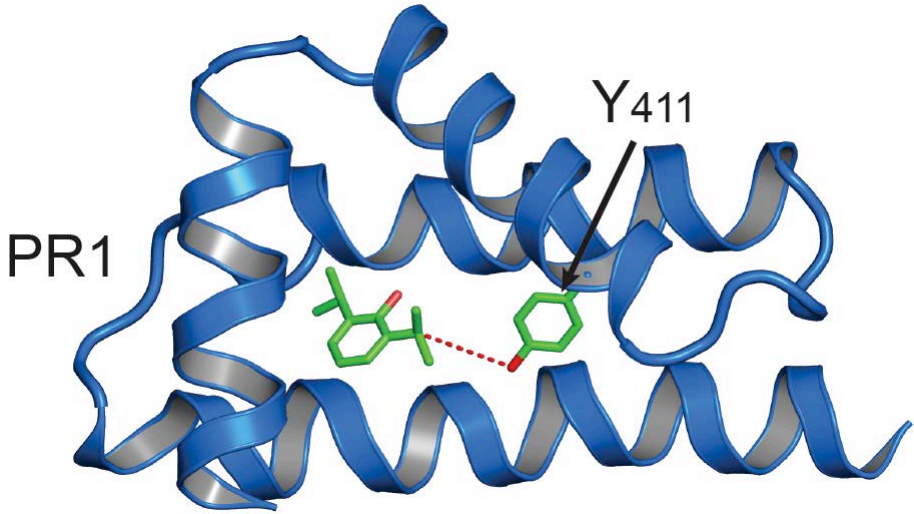
Properties of *ortho*-propofol diazirine

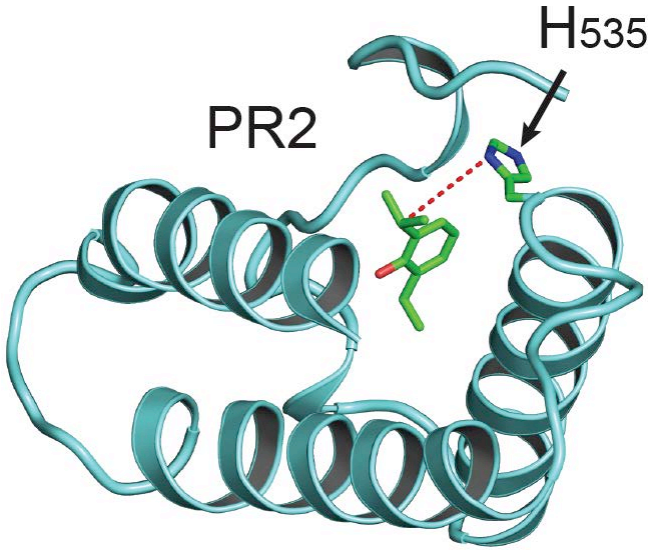


Yip *et al. Nature Chemical Biology* **11,** 715 (2013)

Labelling human serum albumin







Bhattacharya et al. JBC **275,** 38731 (2000)

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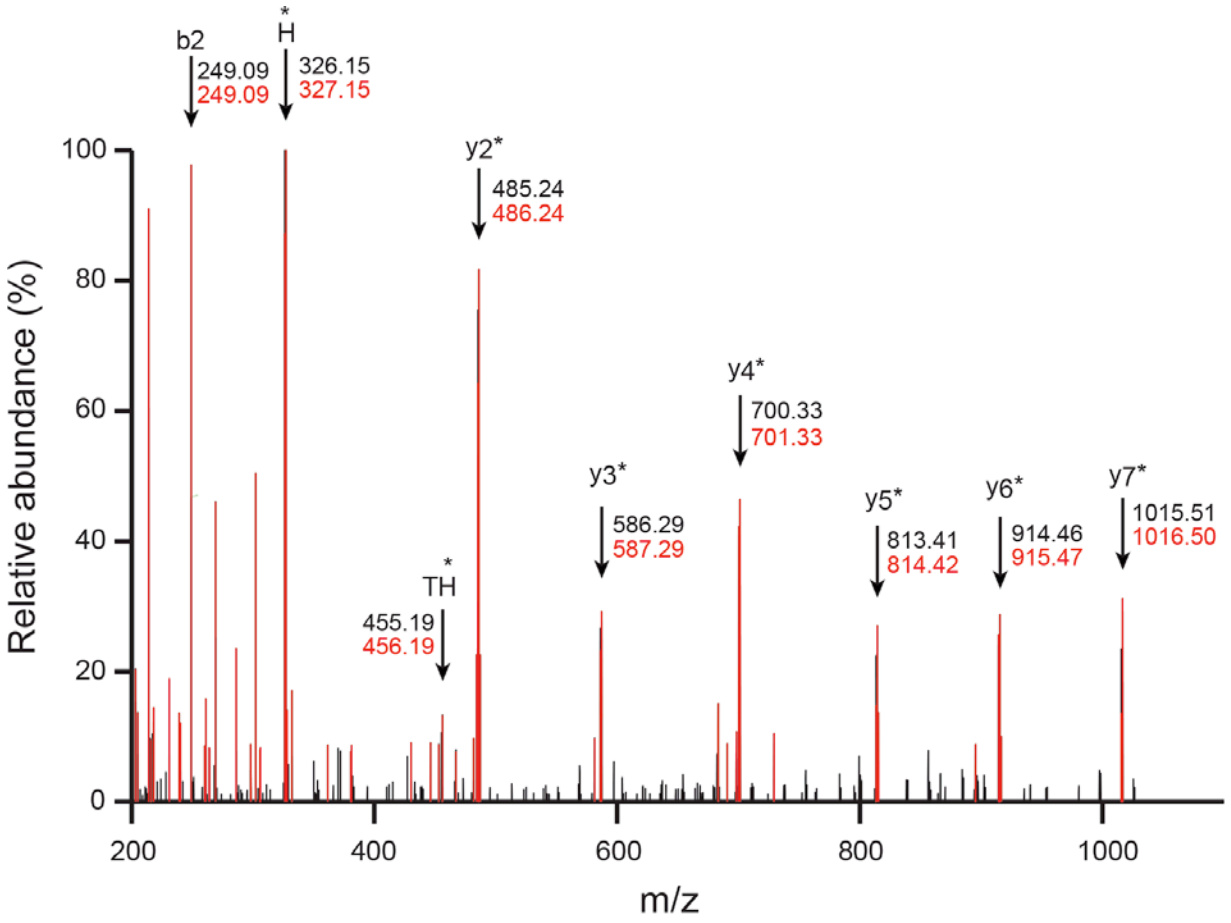
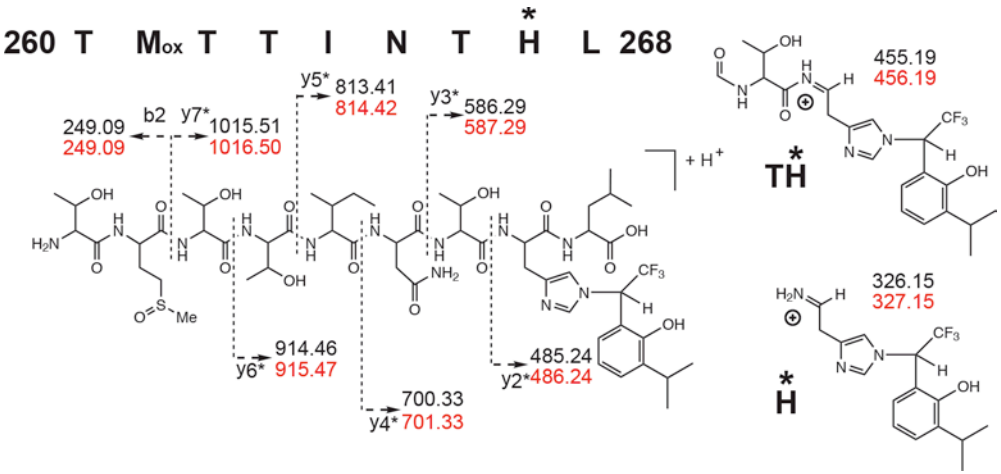
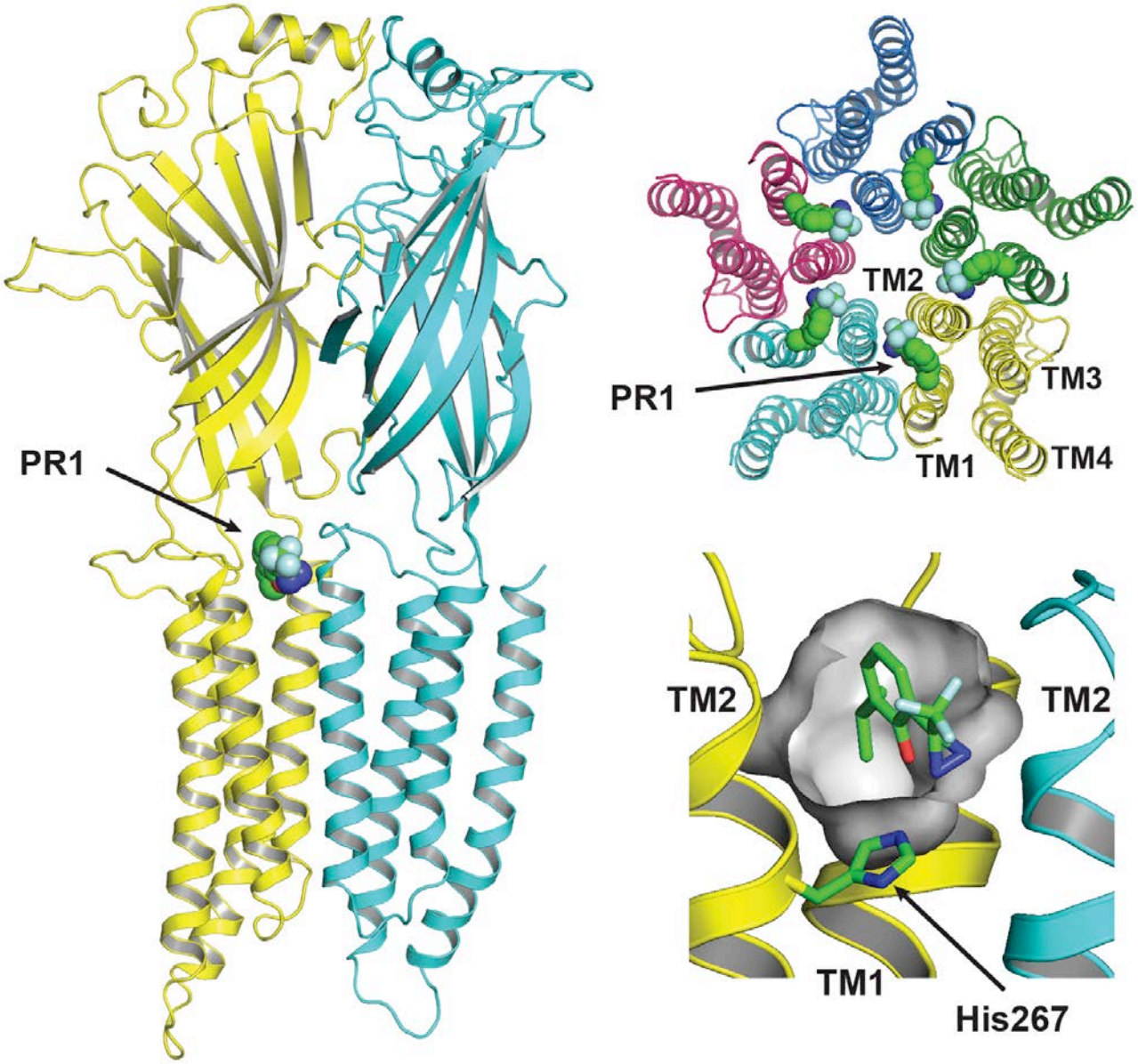


Only a single amino acid is labelled in $GABA_A$ receptors – Histidine 267



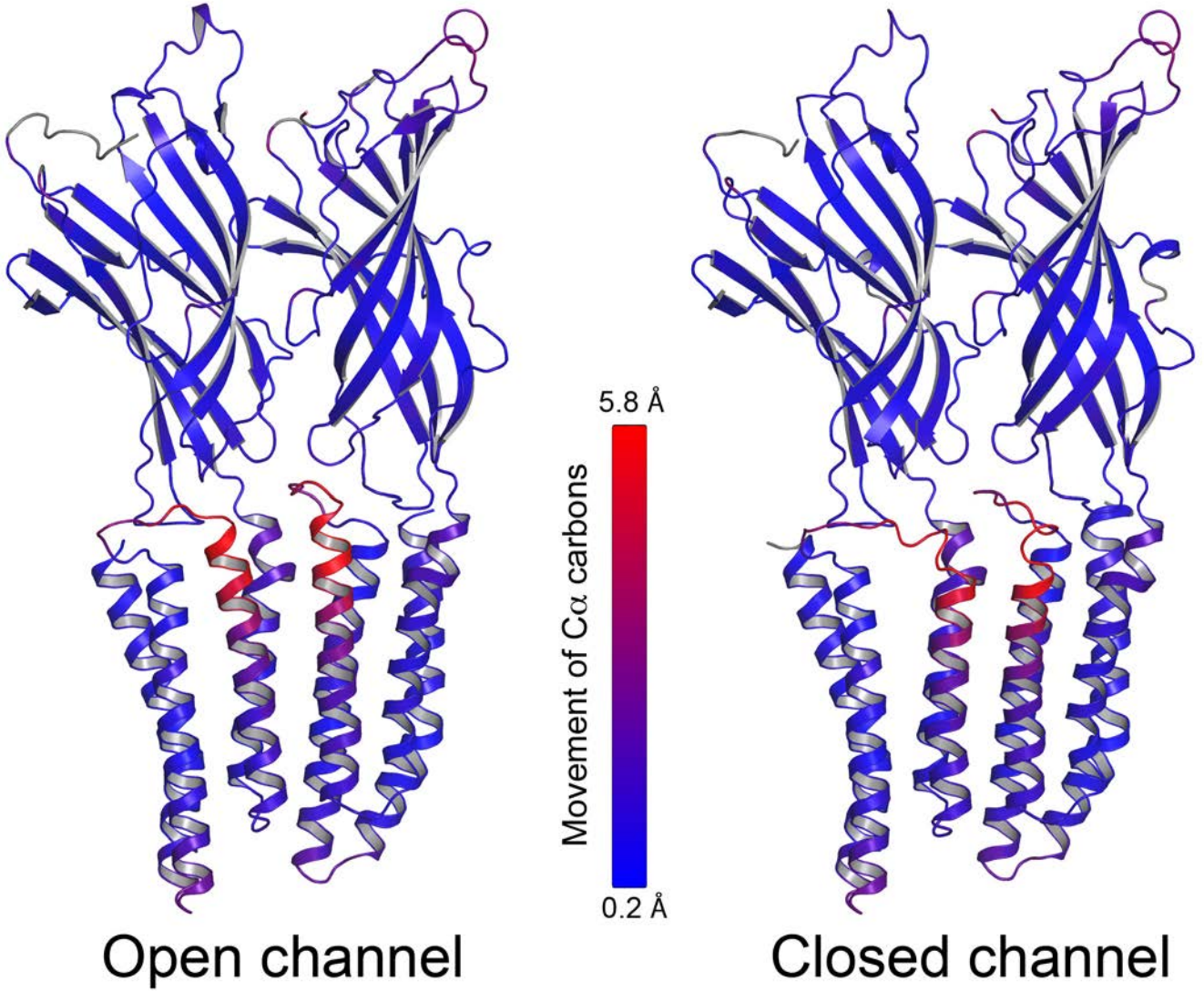
Yip *et al. Nature Chemical Biology* **11,** 715 (2013)

Identification of the binding site



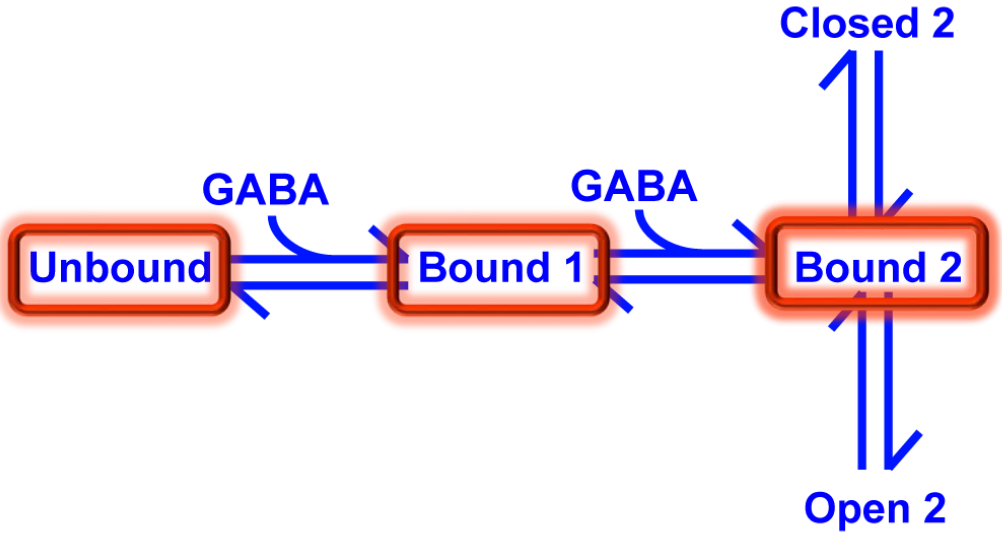
Yip *et al. Nature Chemical Biology* **11,** 715 (2013)

The propofol binding pocket only exists in the open state

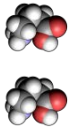


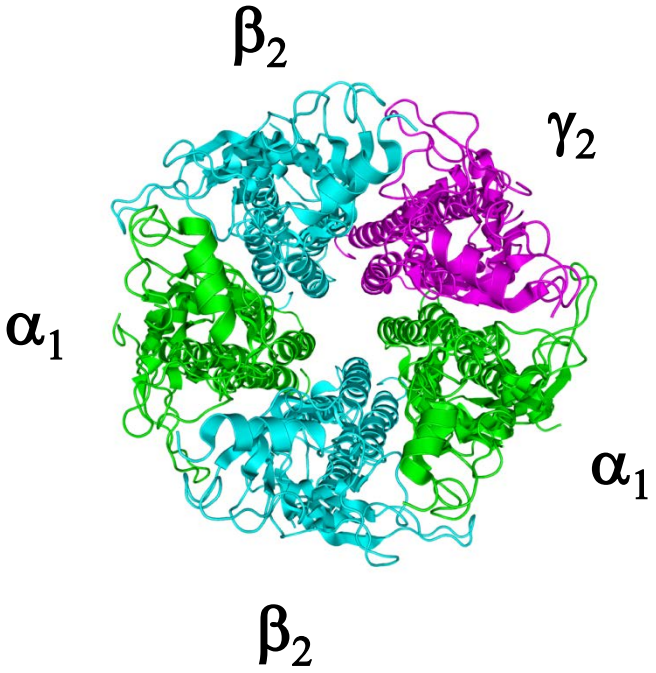
Franks *Anesthesiology* **122,** 787 (2015)

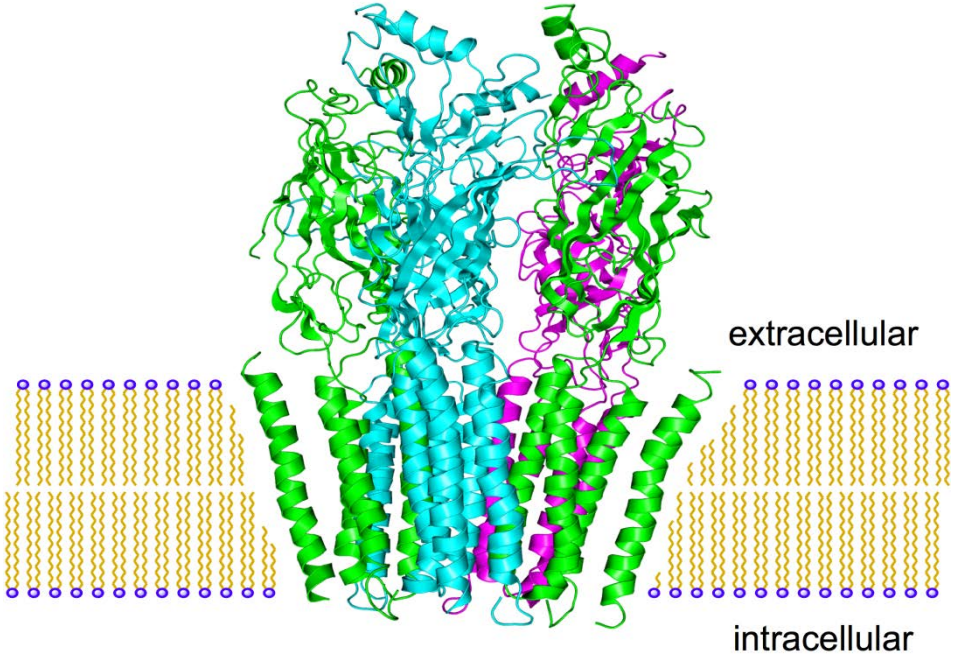
Anaesthetic binding to pre-existing states can explain kinetics

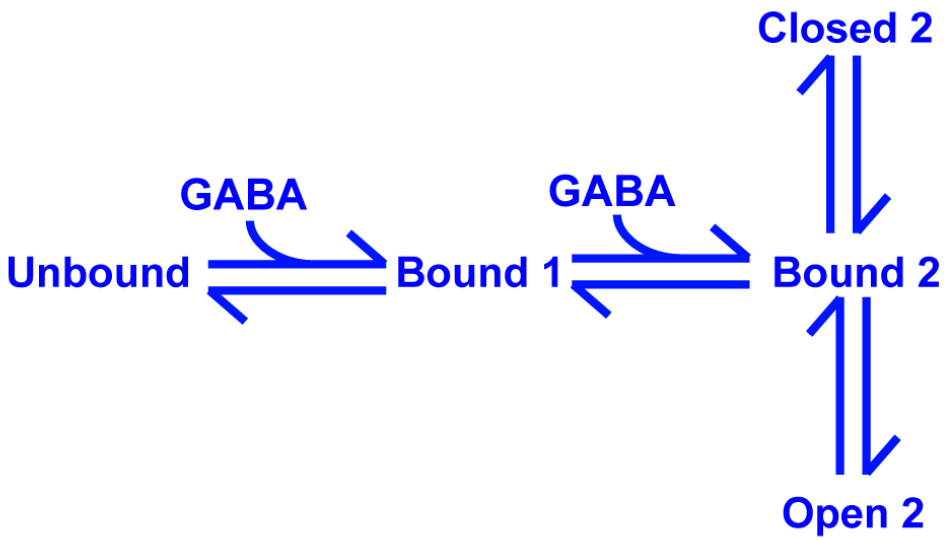


GABA

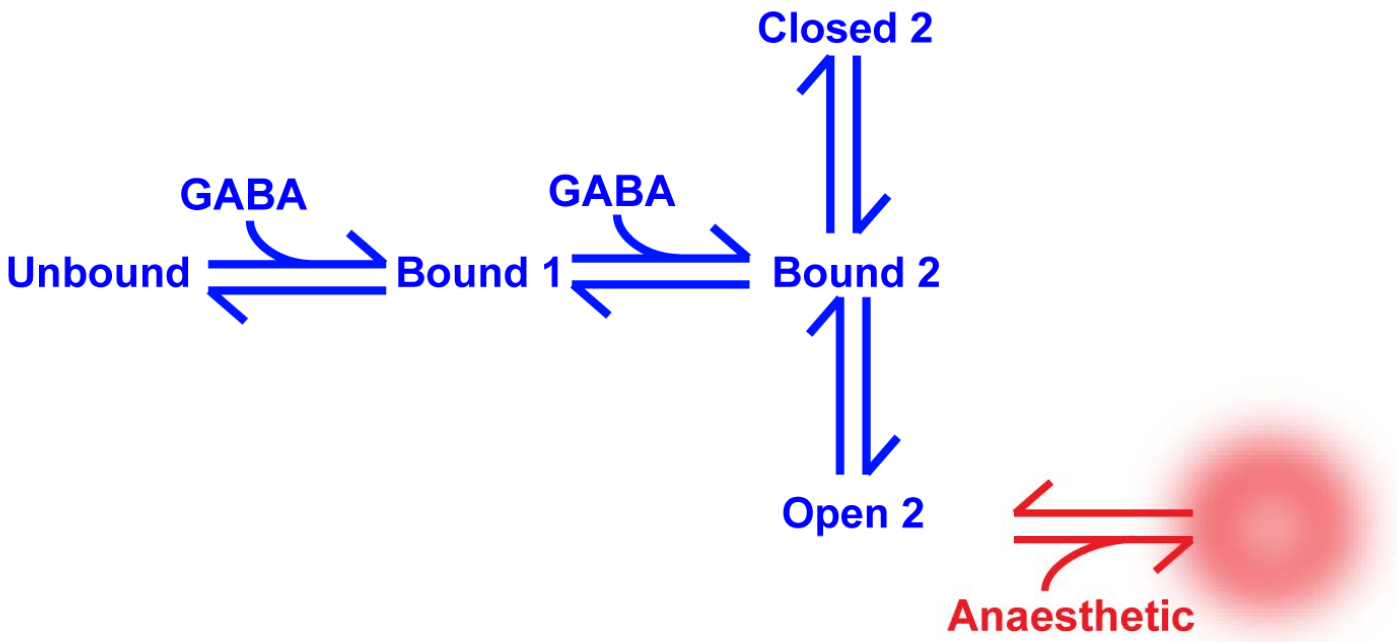




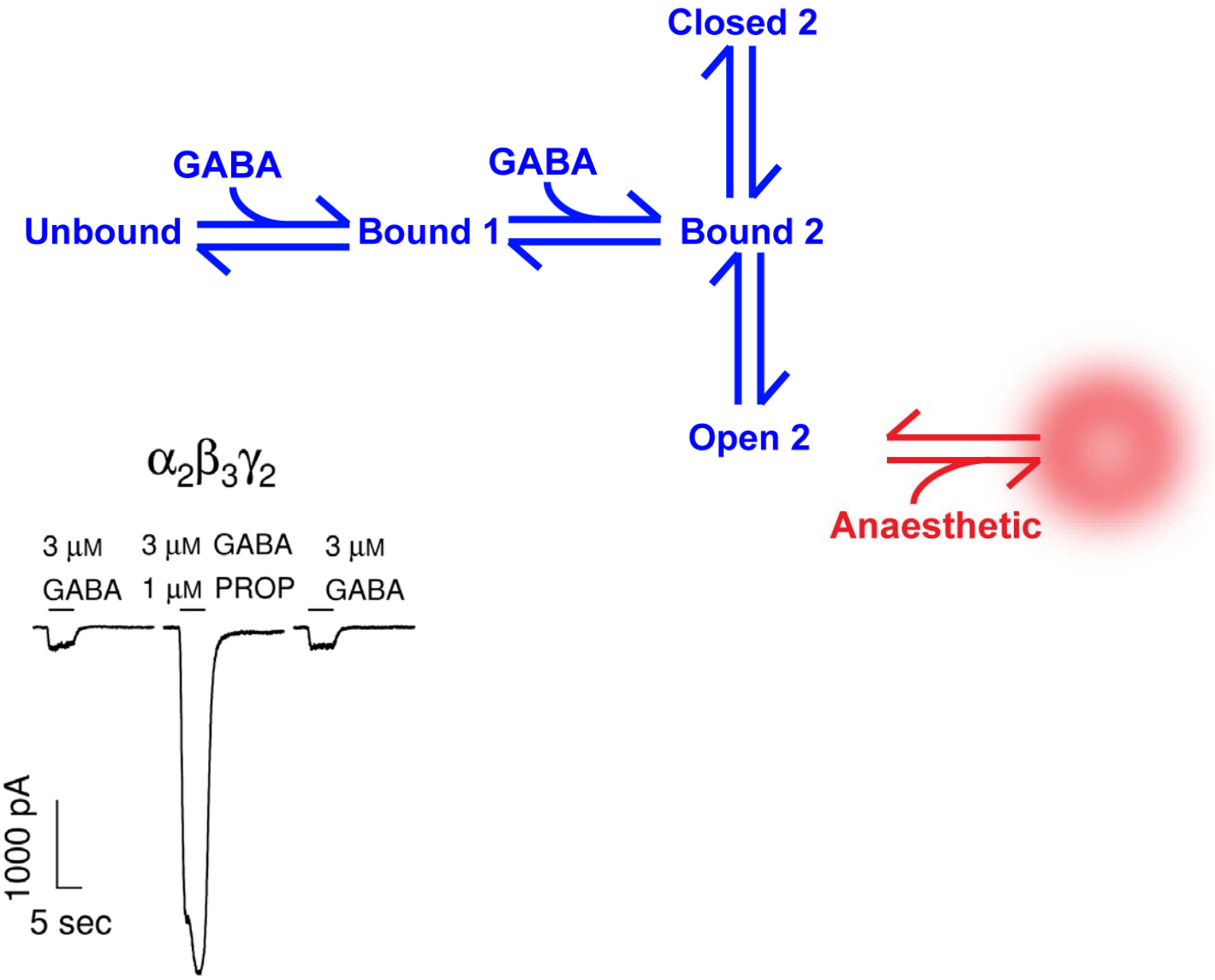




Franks *Nature Rev. Neuroscience* **9,** 370 (2008)



Franks *Nature Rev. Neuroscience* **9,** 370 (2008)



Franks *Nature Rev. Neuroscience* **9,** 370 (2008)

How do anaesthetics act at the network level?

ARTICLES

nature neuroscience

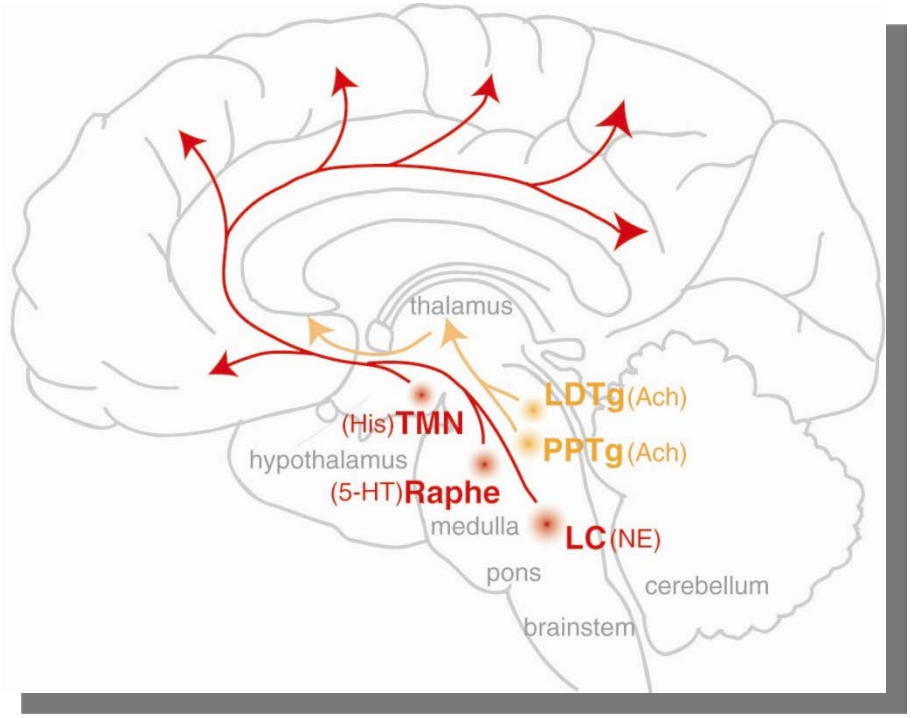
Neuronal ensembles sufficient for recovery sleep and the sedative actions of α_2 adrenergic agonists

Zhe Zhang[1,3], Valentina Ferretti[1,3], İlke Güntan[1], Alessandro Moro[1], Eleonora A Steinberg[1], Zhiwen Ye[1], Anna Y Zecharia[1], Xiao Yu[1], Alexei L Vyssotski[2], Stephen G Brickley[1], Raquel Yustos[1], Zoe E Pillidge[1], Edward C Harding[1], William Wisden[1] & Nicholas P Franks[1]

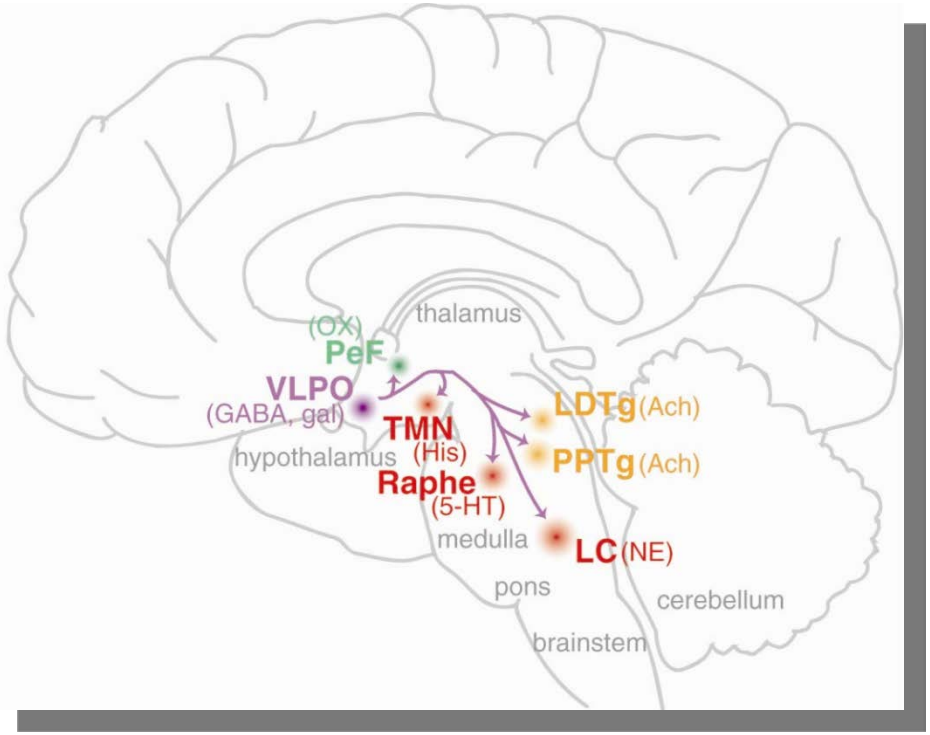
Do sedatives engage natural sleep pathways? It is usually assumed that anesthetic-induced sedation and loss of righting reflex (LORR) arise by influencing the same circuitry to lesser or greater extents. For the α2 adrenergic receptor agonist dexmedetomidine, we found that sedation and LORR were in fact distinct states, requiring different brain areas: the preoptic hypothalamic area and locus coeruleus (LC), respectively. Selective knockdown of α2A adrenergic receptors from the LC abolished dexmedetomidine-induced LORR, but not sedation. Instead, we found that dexmedetomidine-induced sedation resembled the deep recovery sleep that follows sleep deprivation. We used TetTag pharmacogenetics in mice to functionally mark neurons activated in the preoptic hypothalamus during dexmedetomidine-induced sedation or recovery sleep. The neuronal ensembles could then be selectively reactivated. In both cases, non-rapid eye movement sleep, with the accompanying drop in body temperature, was recapitulated. Thus, α2 adrenergic receptor–induced sedation and recovery sleep share hypothalamic circuitry sufficient for producing these behavioral states.

Zhang *et al. Nature Neurosci* **18,** 553 (2015)

Neuronal pathways of sleep and arousal

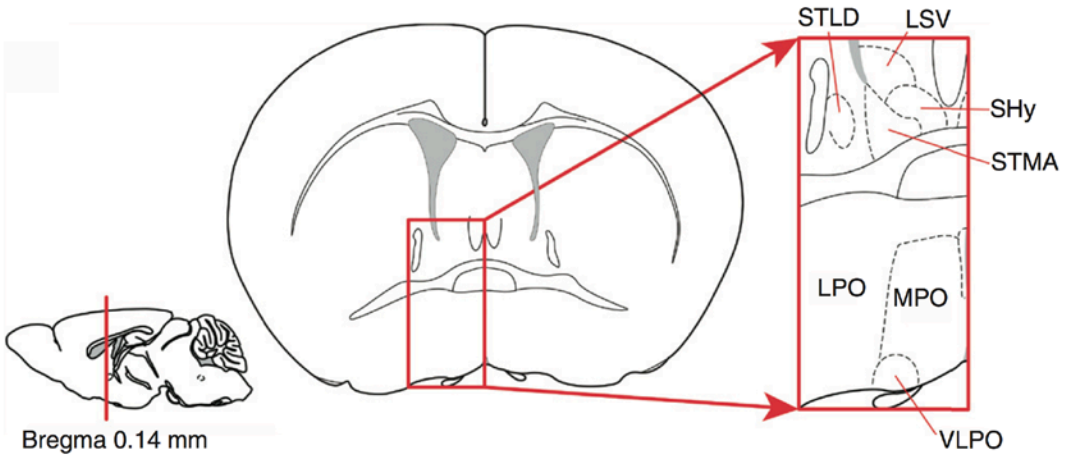


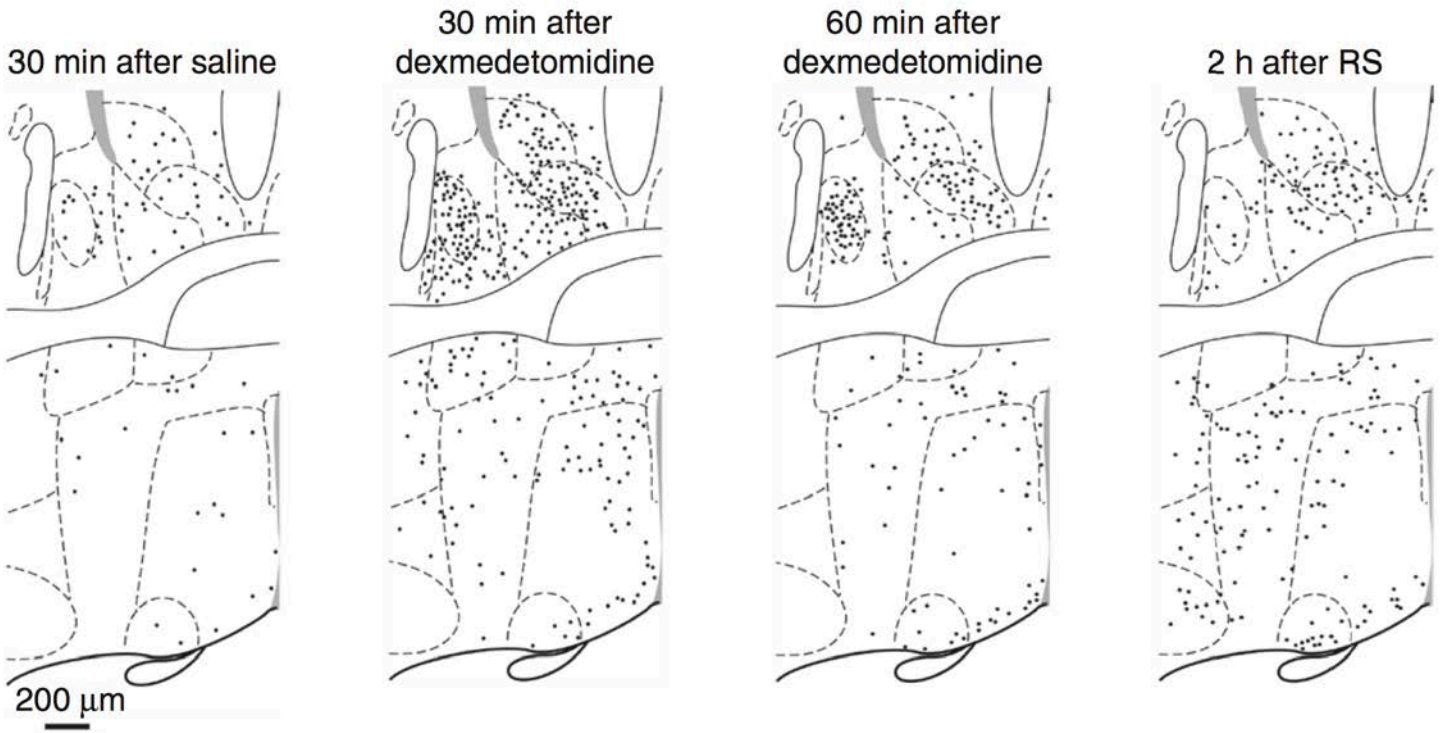
The ascending arousal system in the brain stem, hypothalamus and basal forebrain keeps the neocortex alert & aroused



Activation of the sleep-promoting systems in the hypothalamus and basal forebrain silences these nuclei and promotes sleep

cFOS is upregulated during sedation and deep sleep





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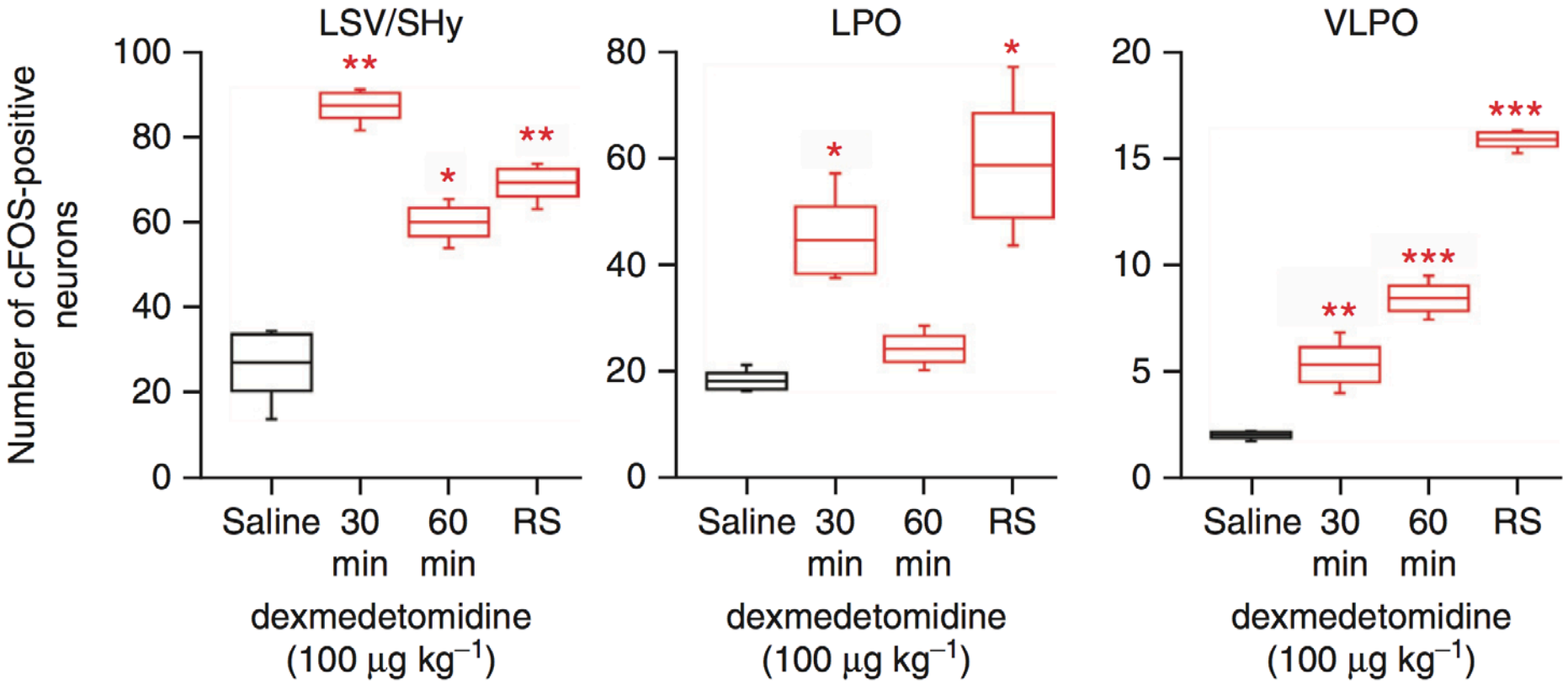
cFOS is upregulated during sedation and deep sleep



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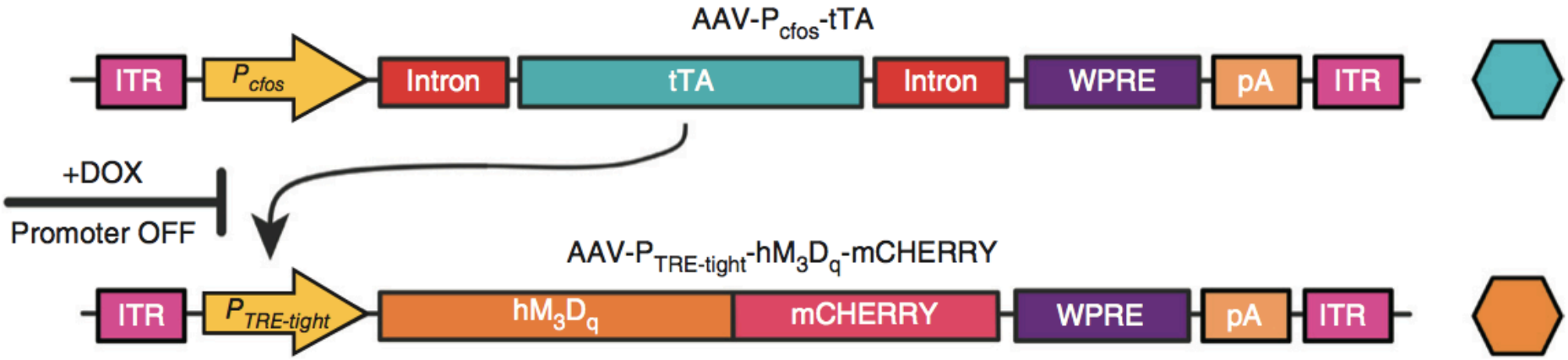
TetTagging allows excited neurons to be tagged



Two viruses are injected which co-transfect the neurons

The first drives expression of the Tet Activator through a *cfos* promotor

In the presence of doxycycline, expression of hM_3D_q is blocked

In the absence of doxycycline, the Tet Activator drives expression of hM_3D_q

The hM_3D_q receptor can be subsequently selectively activated by CNO

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TetTagging allows excited neurons to be tagged

AAV transfection period, no hM_3D_q expression

4 weeks on doxycycline – TetTag system locked	OFF Dox	ON Dox

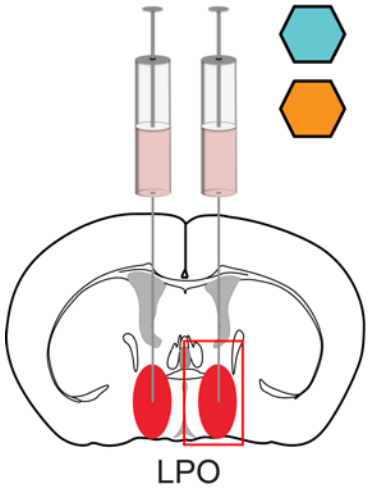
AAV injection

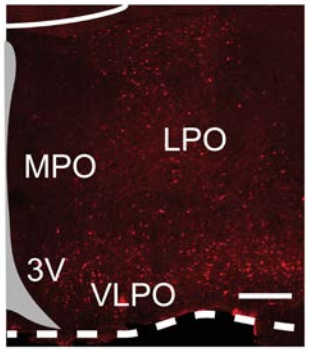
Dex or RS

CNO injection

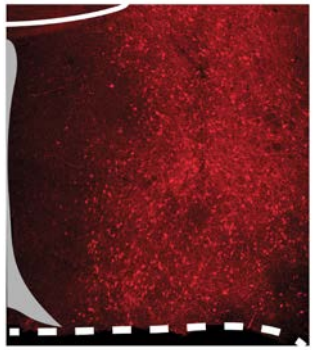
AAV-P_{cfos}-*tTA*

AAV-$P_{TRE\text{-}tight}$-hM_3D_q-*mCHERRY*



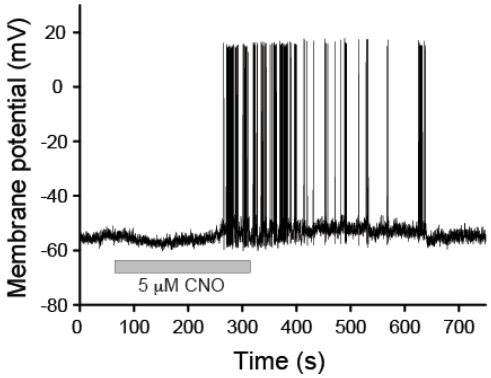


Uninduced (off dox)



Induced by dexmedetomidine

Induced 2 h into recovery sleep



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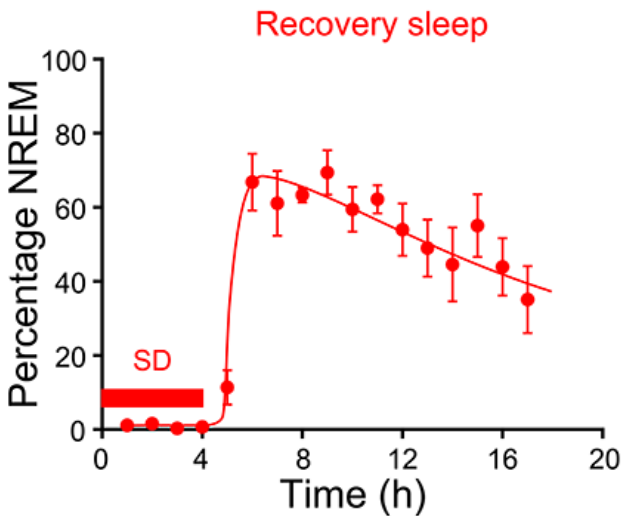
Recovery sleep



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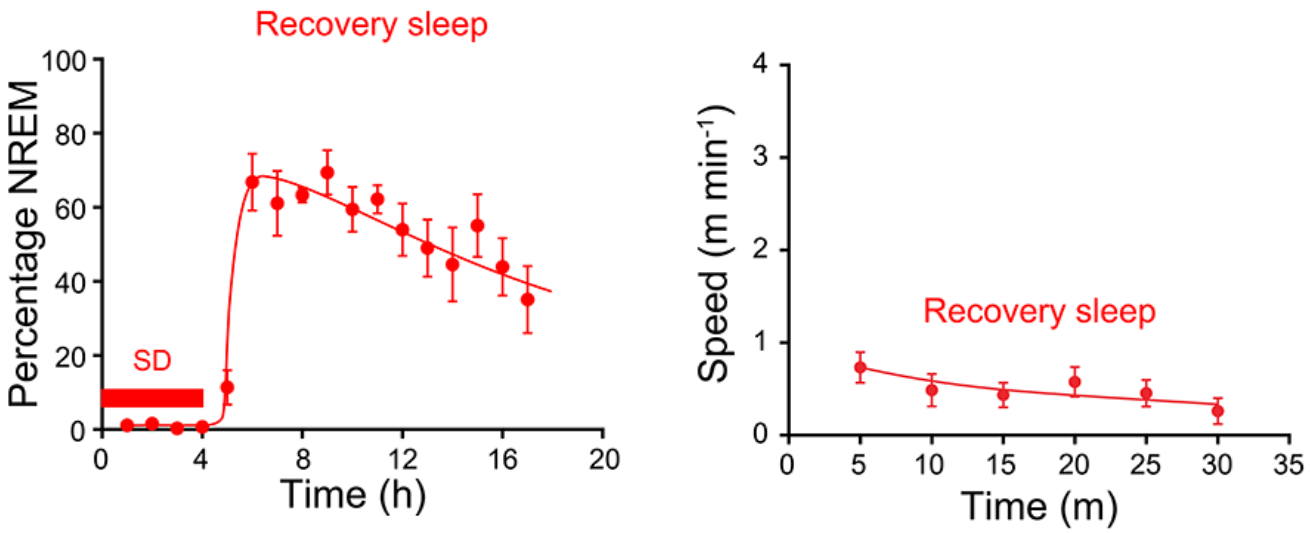
Recovery sleep



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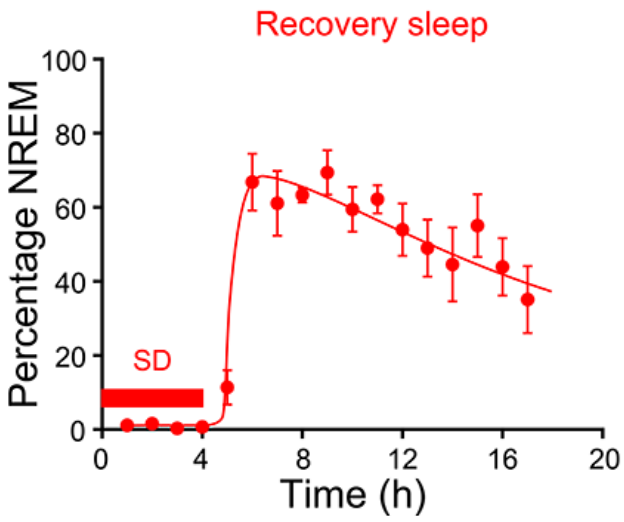
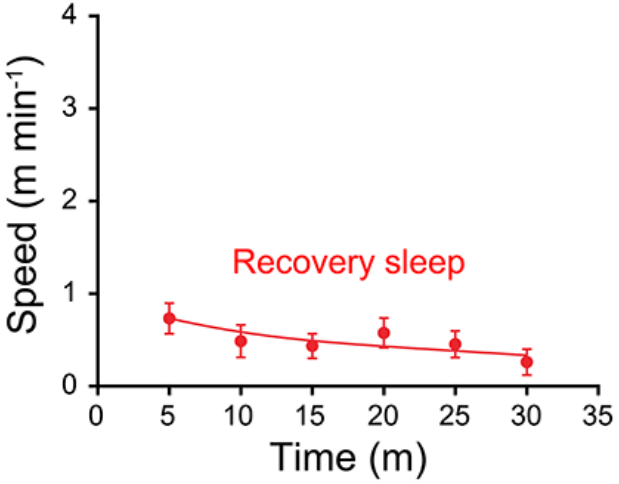
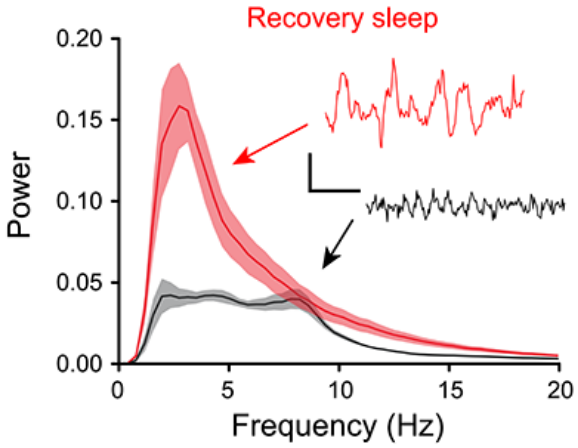
Recovery sleep







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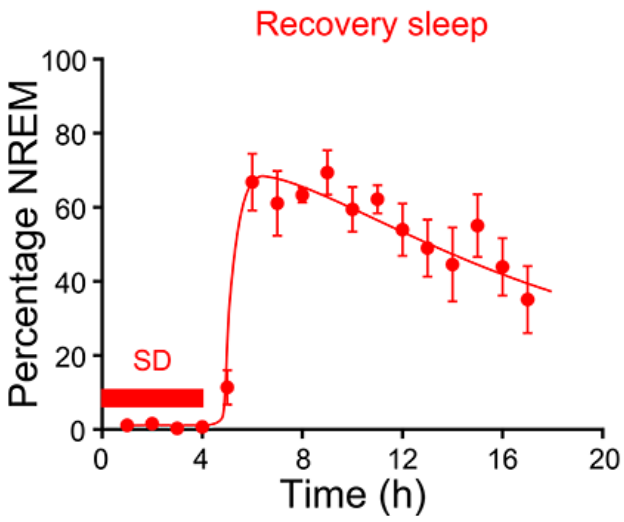
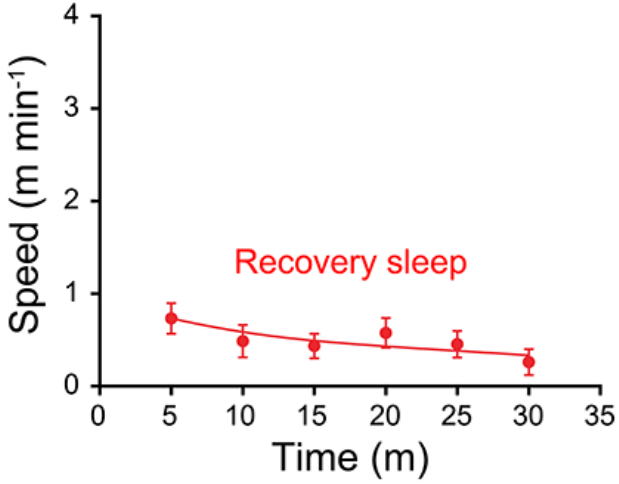
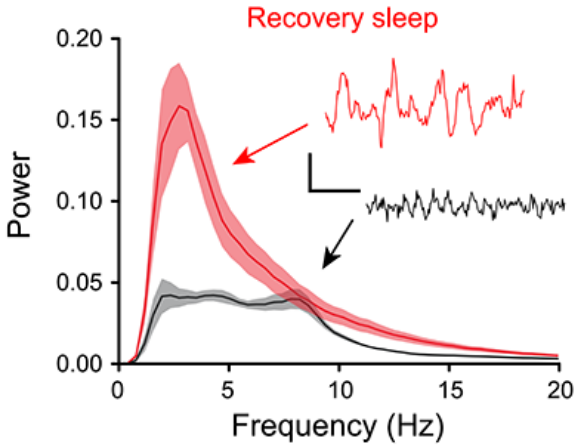
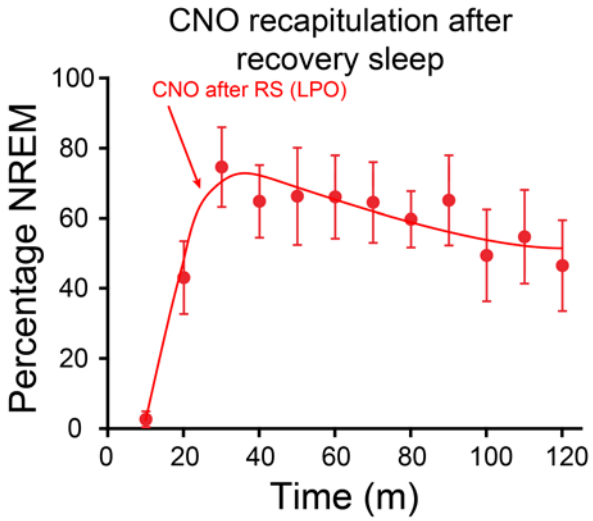
Reactivation of excited neurons recapitulates behaviour









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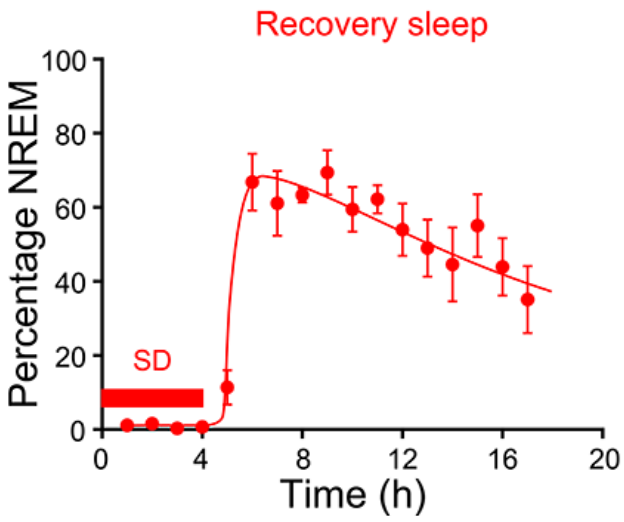

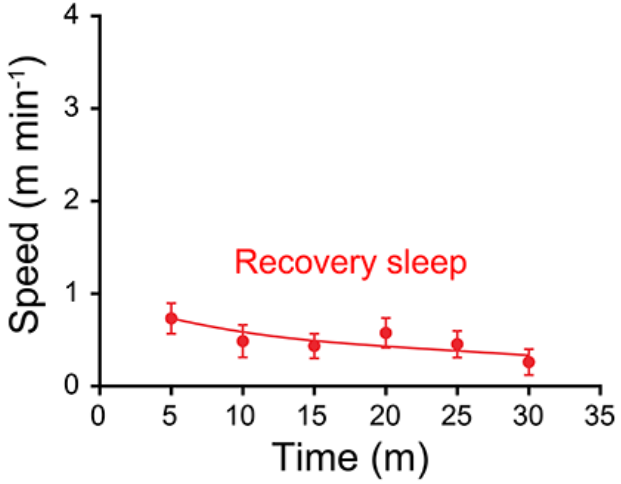

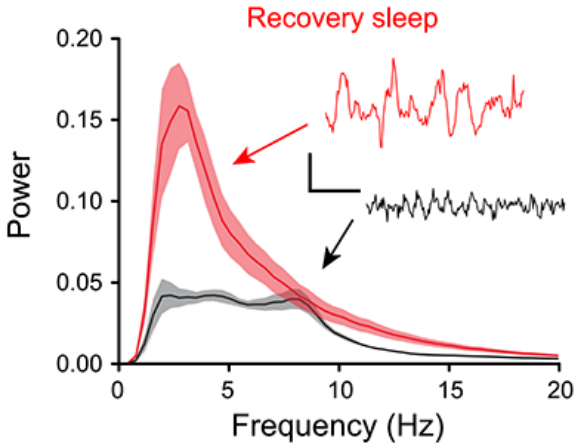

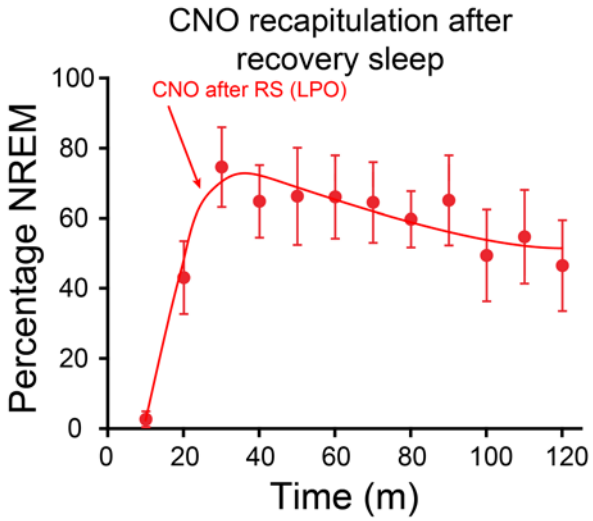

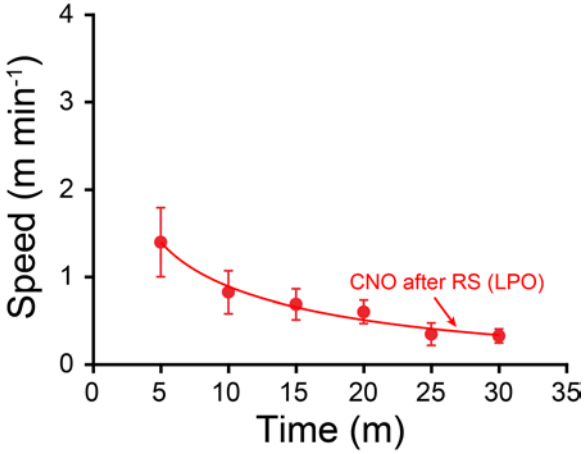

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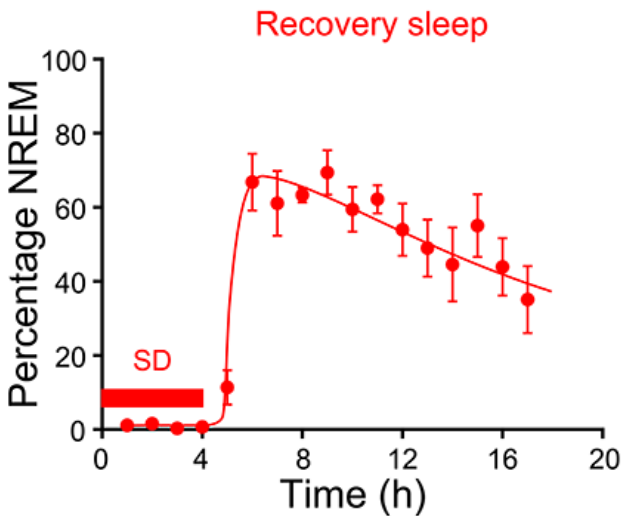

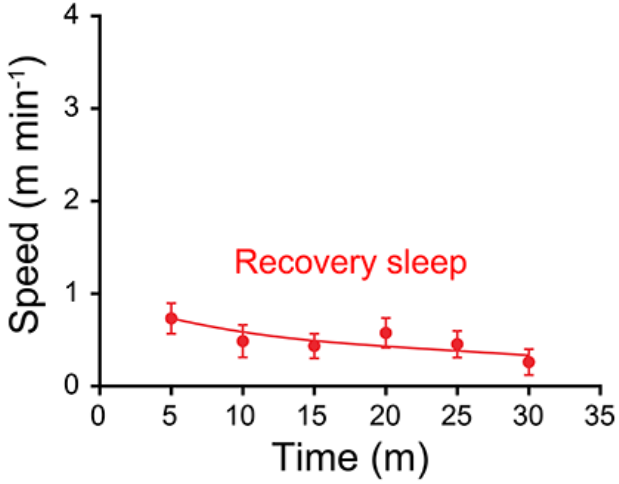

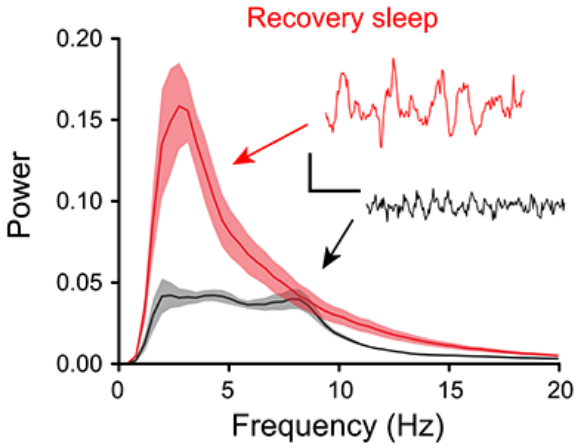

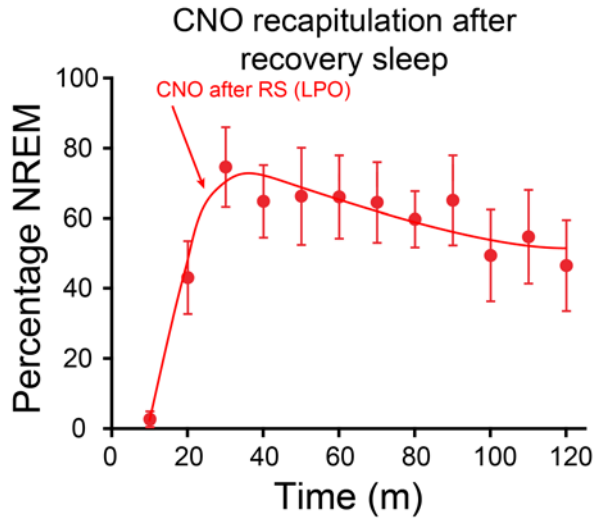

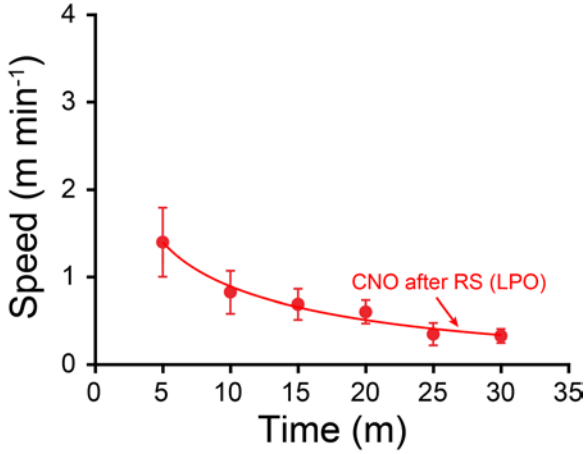

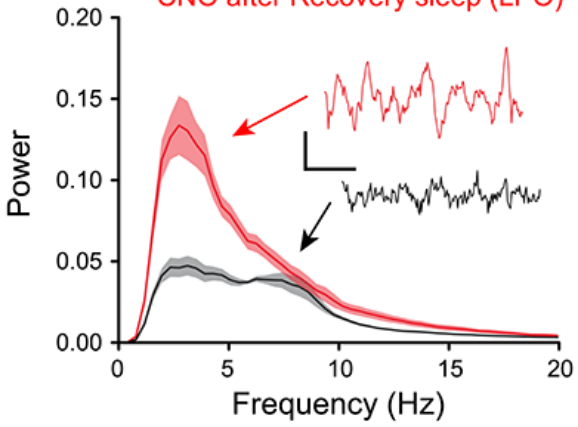

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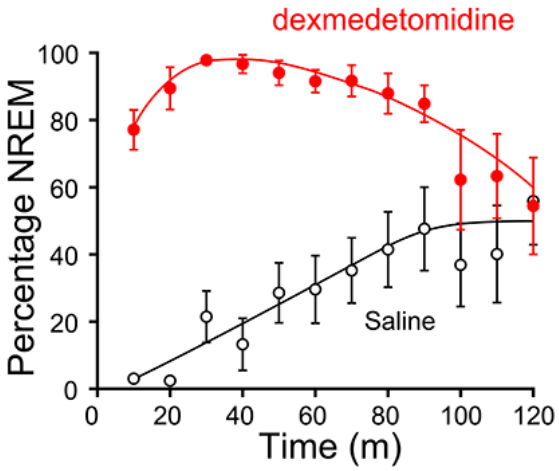
Dexmedetomidine sedation



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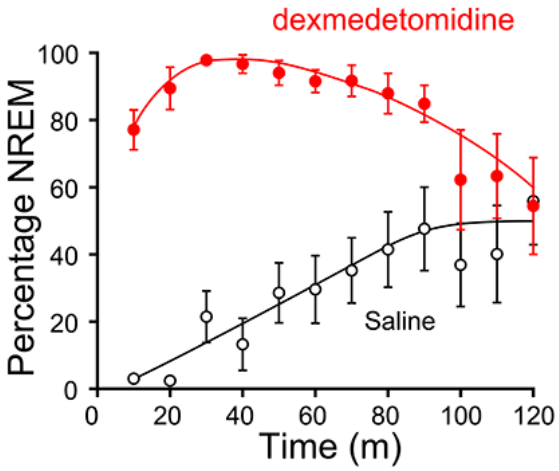

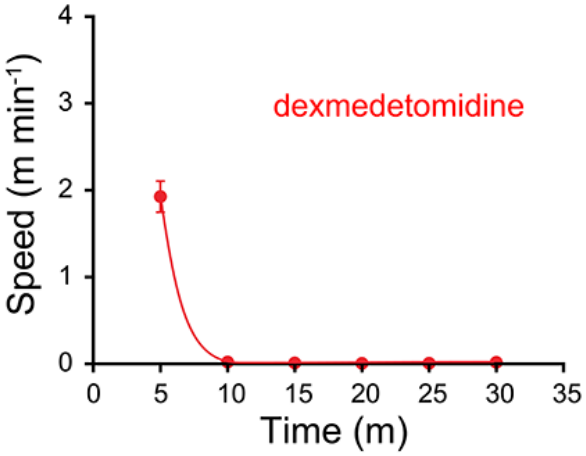

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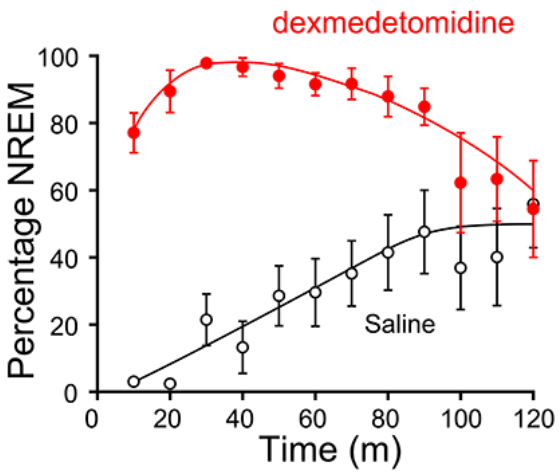
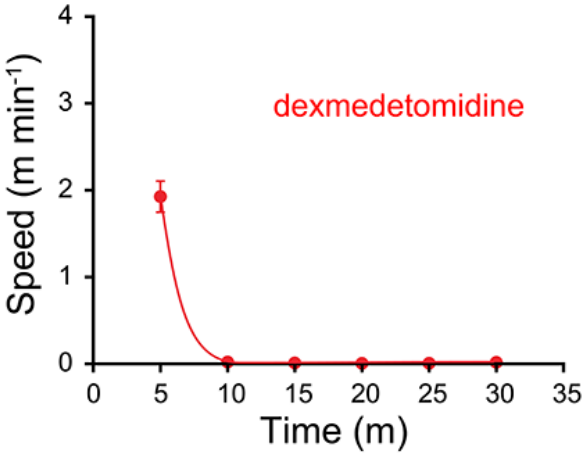
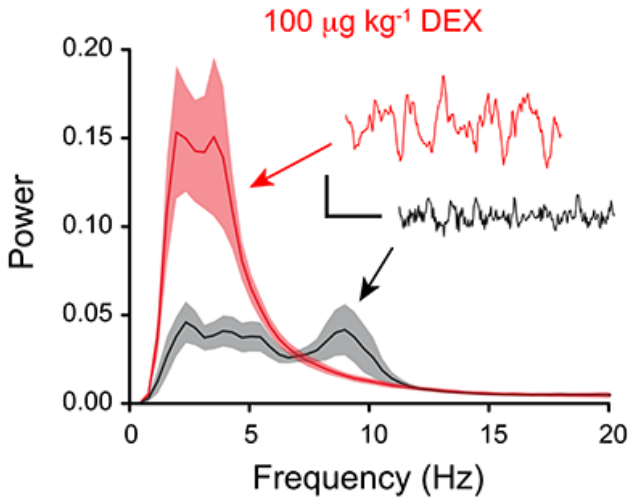
Dexmedetomidine sedation







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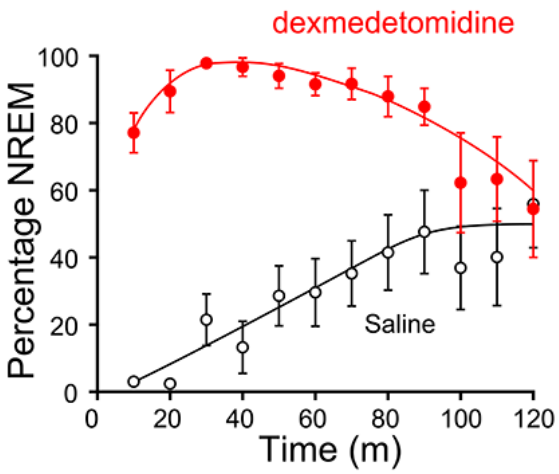

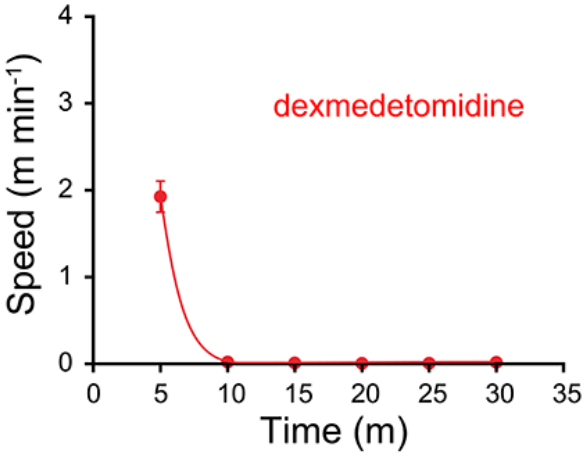

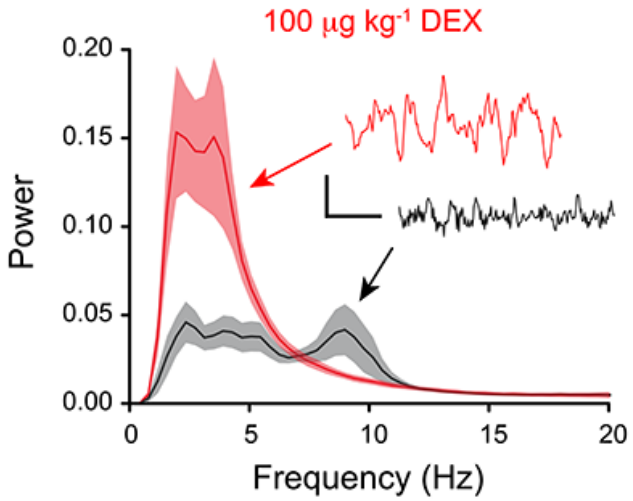

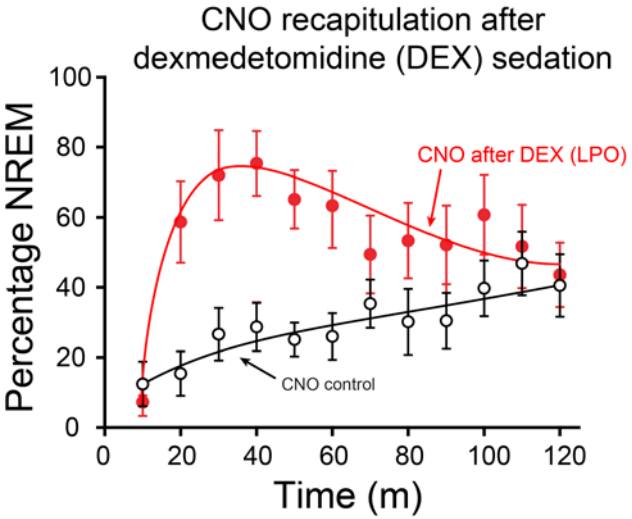

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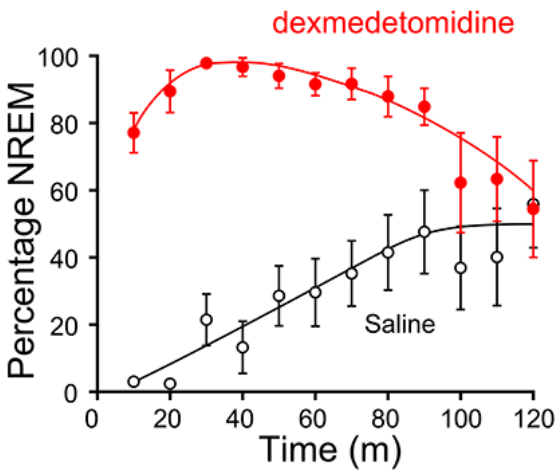

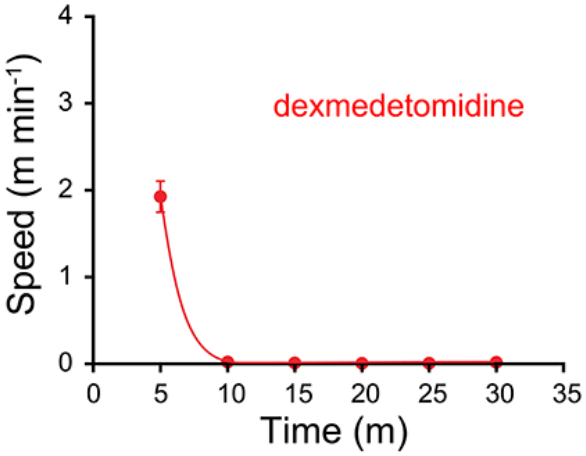

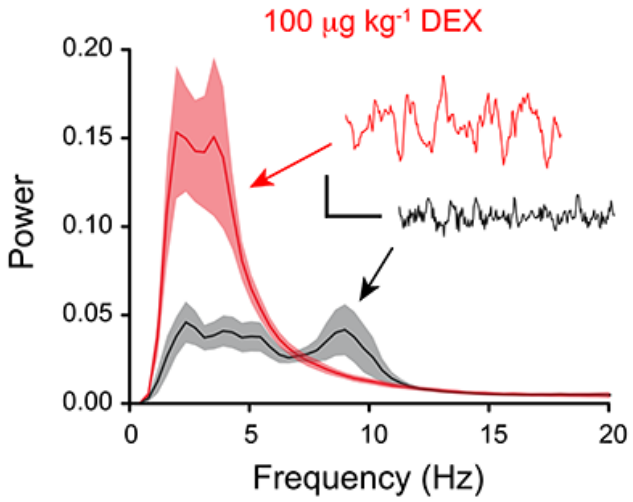

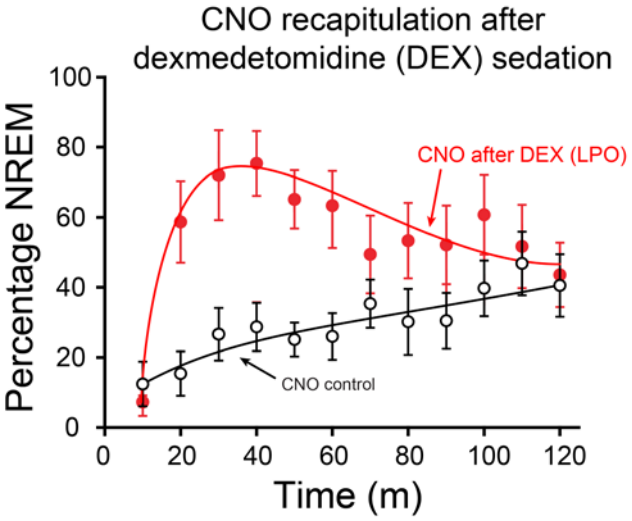

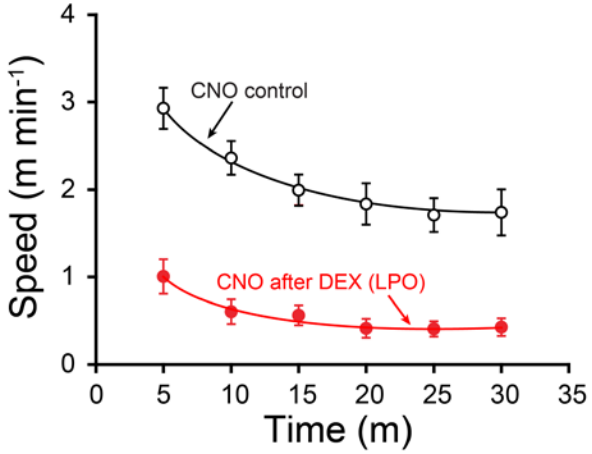

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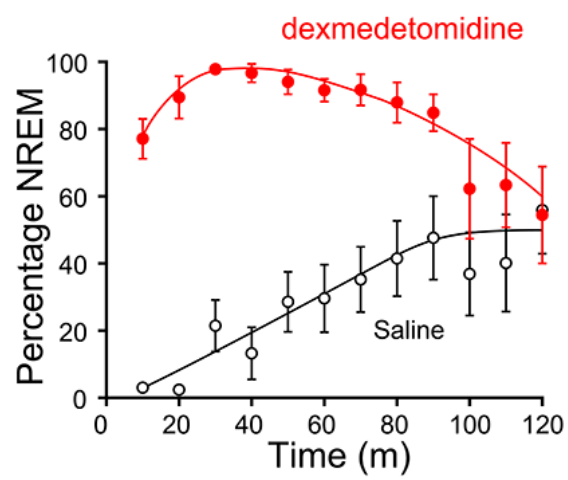

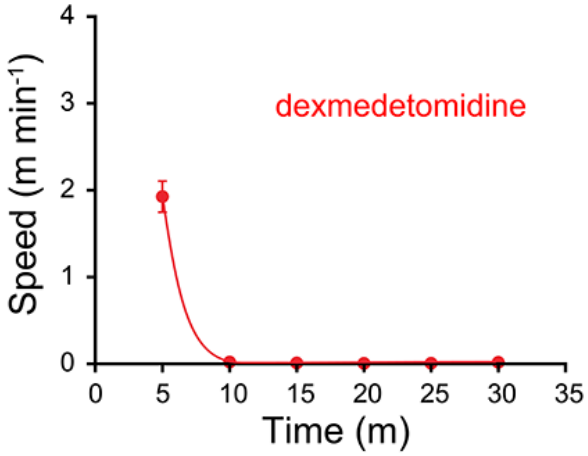

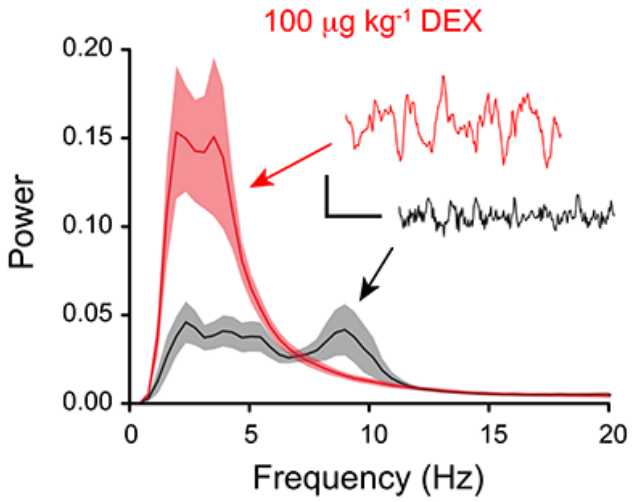

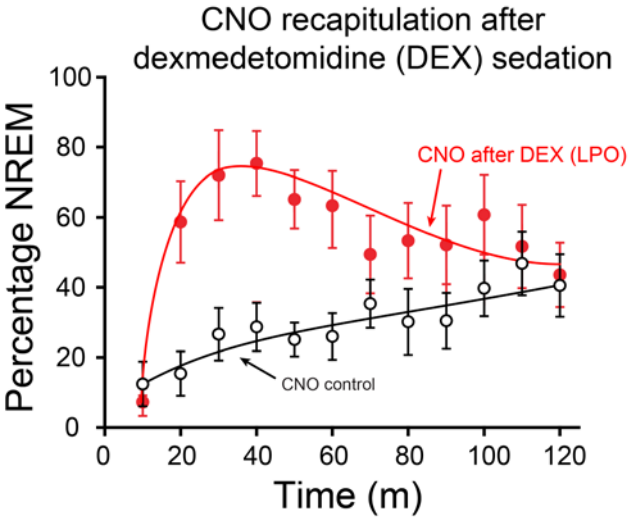

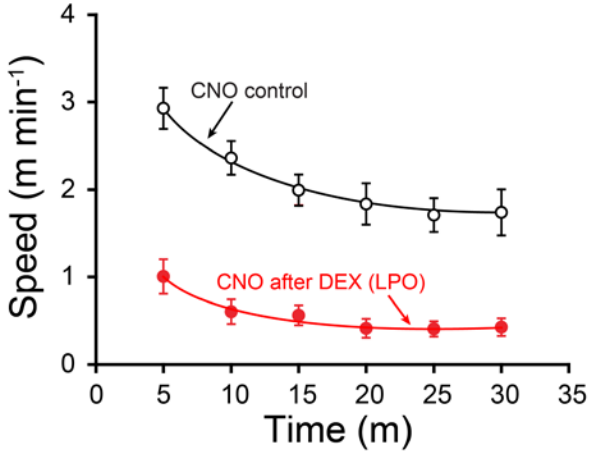

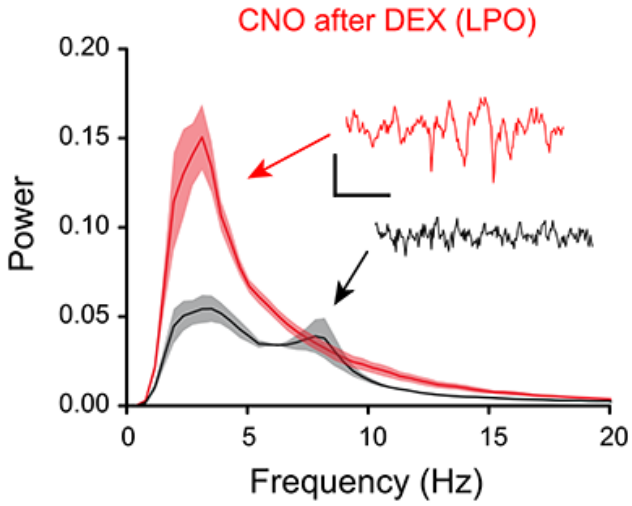

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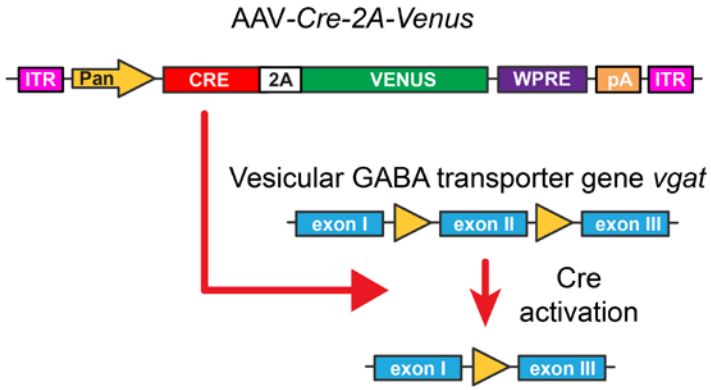
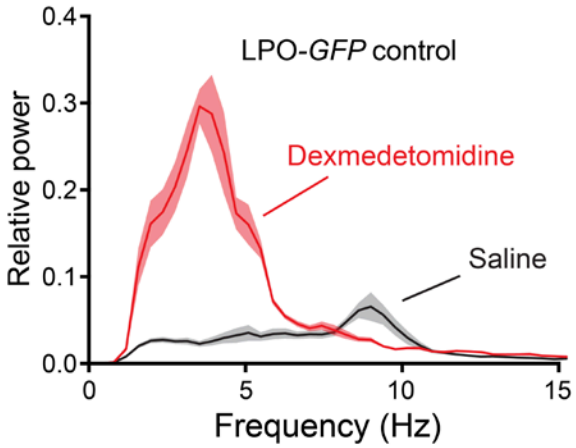
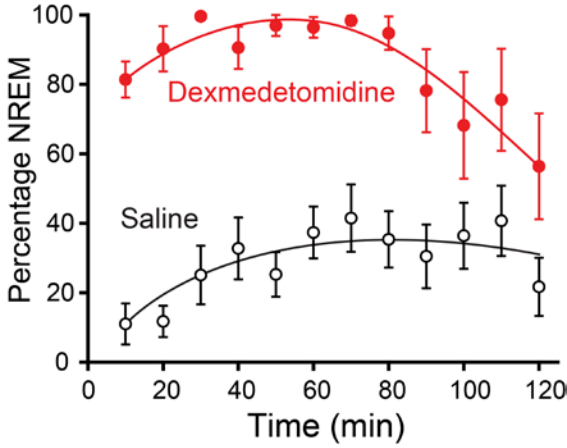
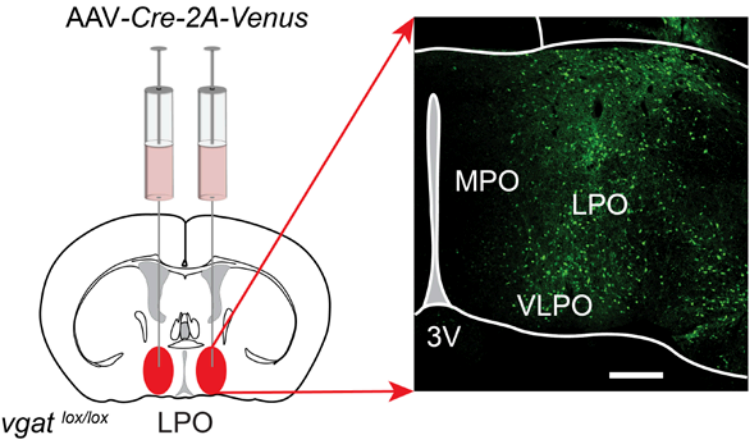
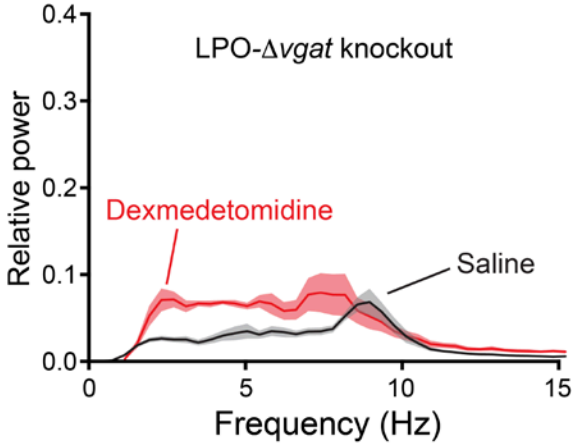
Role of GABA release in dexmedetomidine sedation

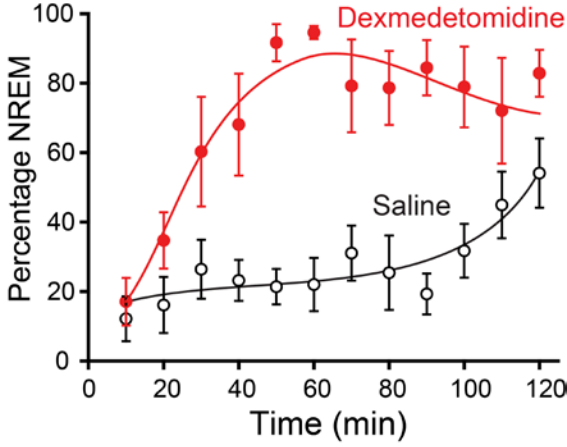












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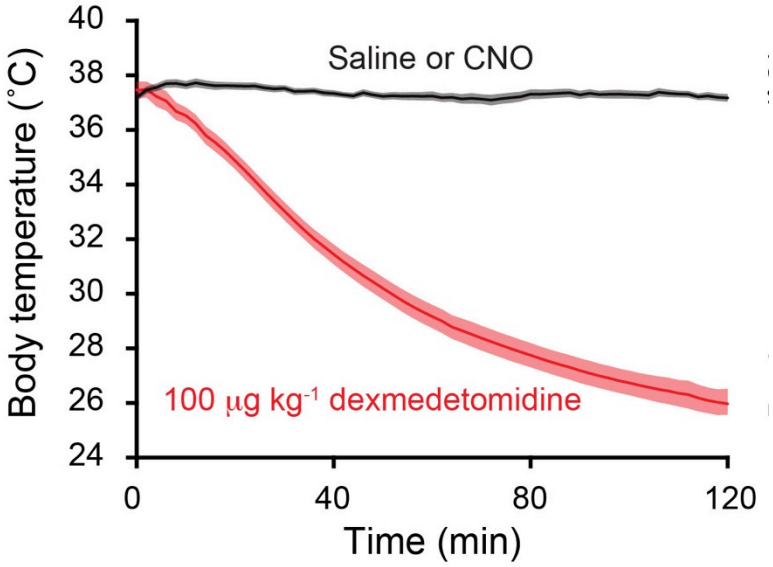
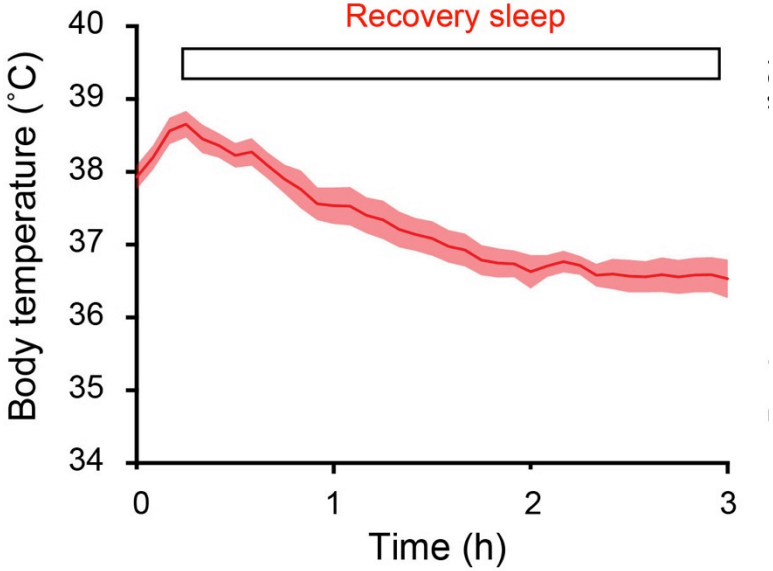
Reactivation of excited neurons recapitulates hypothermia





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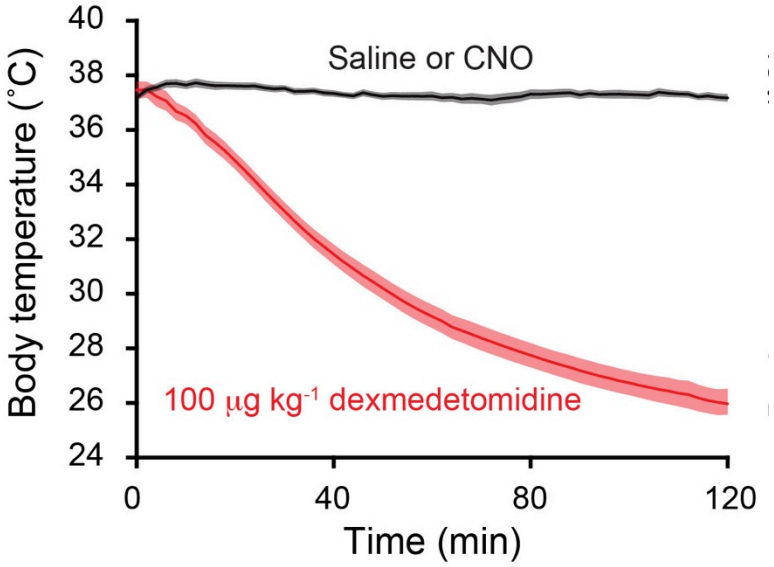

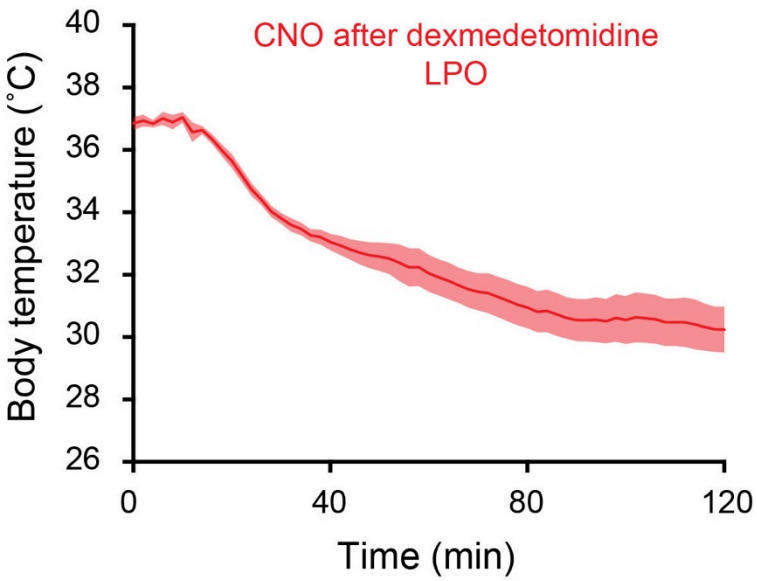

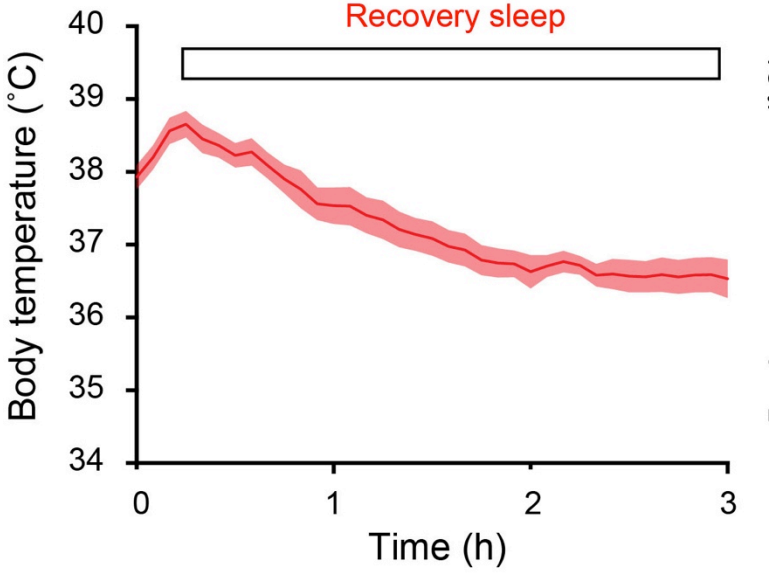

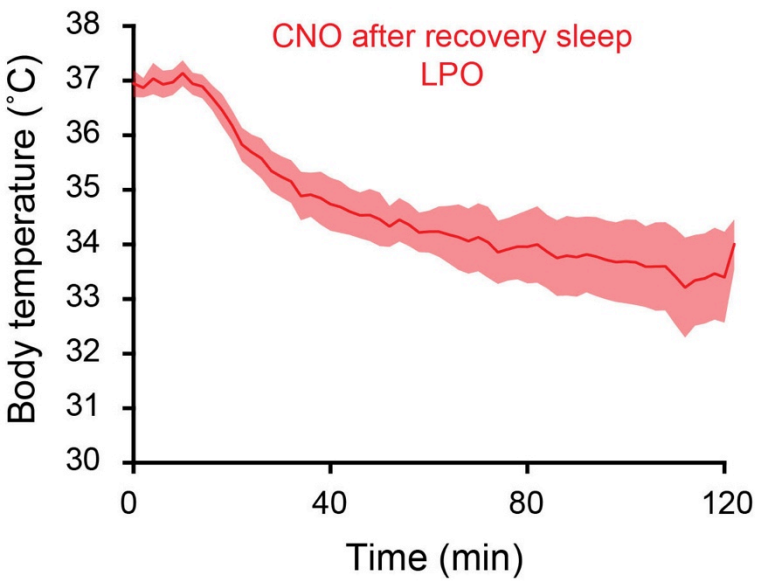

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Summary and conclusions

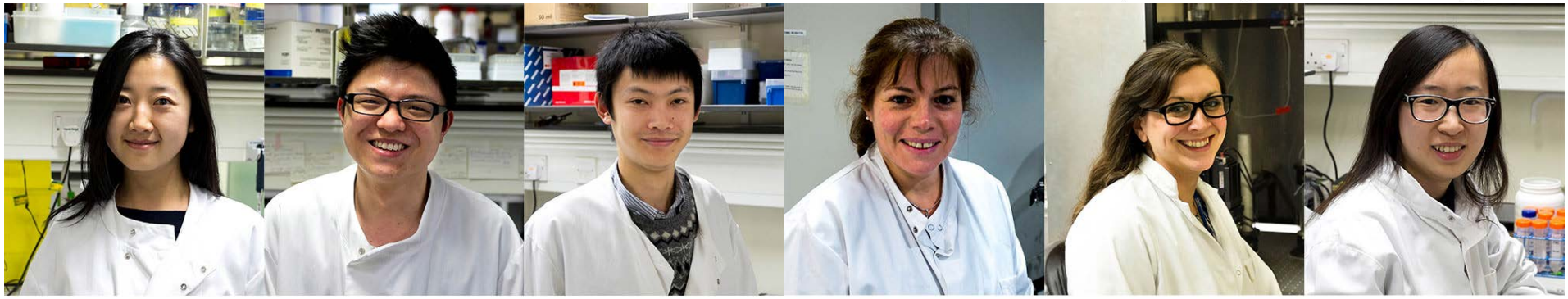
- Anaesthetics act by binding to pre-formed cavities that exist in some conformational states of the protein, but not others.
- Propofol acts mainly on the $GABA_A$ chloride channel and binds to a site at the interface between the extracellular and transmembrane domains..
- Dexmedetomidine induces a state closely resembling natural sleep.
- The sedation by dexmedetomidine requires activation of a small group of neurons in the pre-optic hypothalamus.
- The same neurons, or at least an overlapping ensemble, are responsible for dexmedetomidine-induced hypothermia

Acknowledgements

Bill Wisden & Stephen Brickley and:

Rowan Baker, Valentina Ferretti, Fabia Fricke, Cigdem Gelegen, Ed Harding, Cat Houston

Lily Le, Alessandro Moro, Chris Edge, Ed Smith, Elly Steinberg, Dave Uygun



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Thanks for listening

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