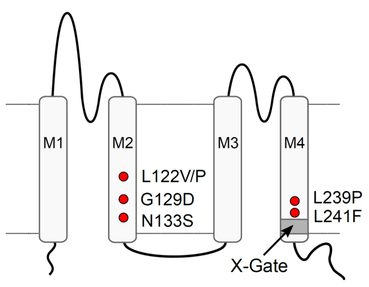
Defective X-Gating of TASK-1 in a Novel Channelopathy Associated with Sleep Apnea

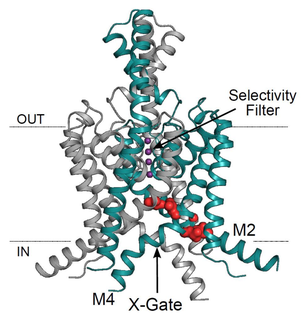
Janina Sörmann[1,2], Marcus Schewe[3], Peter Proks[1,2], Thibault Jouen-Tachoire[1,2,4,5], Shanlin Rao[6], Karin E.J. Rödström[7], Thomas Müller[8], Thomas Baukrowitz[3], Matthew E. Hurles[9], Caroline F. Wright[10] and Stephen J. Tucker[1,2,5]

1: Dept Physics, University of Oxford, UK, 2: Kavli Institute for Nanoscience Discovery, University of Oxford, UK, 3: Institute of Physiology, Kiel University, Kiel, Germany, 4: Dept Pharmacology, University of Oxford, UK, 5: OXION Initiative in Ion Channels and Disease, 6: Dept Biochemistry, University of Oxford, UK, 7: Centre for Medicines Discovery, University of Oxford, UK, 8: Bayer AG, Wuppertal, Germany, 9: Wellcome Sanger Institute, Cambridge, UK, 10: Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK.

KCNK3 (TASK-1) has a high burden of *de novo* mutations in a novel channelopathy

- *KCNK3* recently identified as one of 28 novel genes with a high burden of *de novo* mutations linked to developmental delay in children (Kaplanis, et al. *Nature,* 2020)
- All probands with mutations in *KCNK3* also had sleep apnea and needed nocturnal oxygen.
- We now have identified 9 probands each heterozygous for one of 6 separate *de novo missense* mutations in *KCNK3*





Probands show a common phenotype:

- **Developmental delay**
- **Sleep apnea** (Nocturnal O_2, Nadir SpO_2: 67-92%, AHI: 2-54)
- Hypotonia and variable structural abnormalities
- Learning difficulties

➢ We termed the disease **Developmental Delay with Sleep Apnea (DDSA)**

TASK-1 implicated in sleep apnea

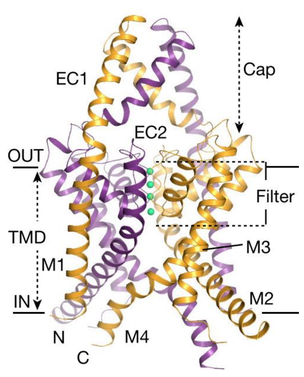
Up to 1 billion people worldwide suffer from **central** or **obstructive sleep apnea.** It occurs when breathing stops repeatedly during sleep.

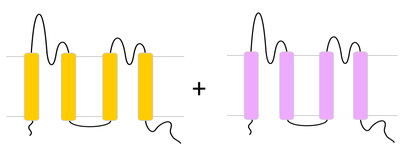
A role for TASK-1 in sleep apnea was proposed because it is expressed in the chemosensitive regions of the brain, carotid body, vascular smooth muscle and hypoglossal motor neurons.

Inhibitors of TASK-1 also in clinical trials for sleep apnea (KOALA trial)

But the evidence is mostly circumstantial. This study now provides the first clear link between TASK-1 and sleep apnea

K2P K^+ channel TASK-1



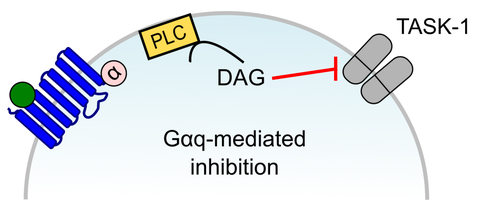


Rödström, et al. (*Nature* 2020)

- Two subunits form one channel
- Can also form 'heteromeric' channels with TASK-3 (*KCNK9*)
- Structure reveals novel lower 'X-gate'
- Regulated by external pH (H^+ inhibition)

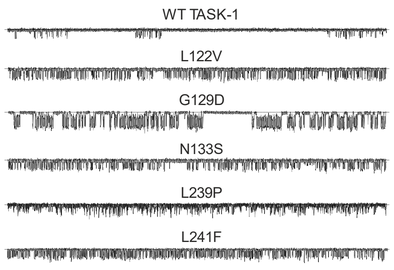
GPCR (Gαq) regulation of TASK-1

- TASK-1 activity also regulated by G-protein coupled receptor (GPCR) pathways
- Gαq regulation involves non-canonical pathway (DAG inhibition)
- Allows integration into different neuronal, metabolic and cellular signalling pathways
- Mutations in M4 (X-gate) are known to affect GPCR regulation

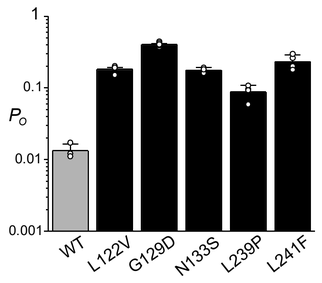


Defective X-gating

DDSA mutant channels have a higher open probability



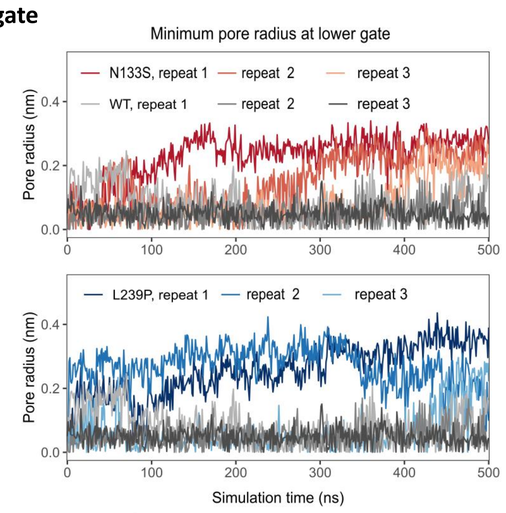
WT TASK-1 has a very low open probability (*Po* ~ 0.02) and the single channel conductance is ~13 pS.



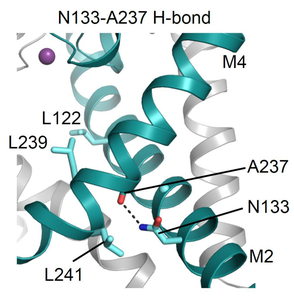
Homomeric DDSA mutants have open probabilities *Po* > 10 times greater than WT TASK-1.

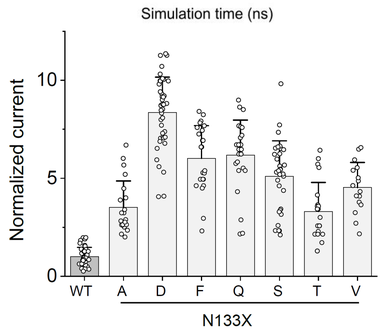
Molecular Dynamics simulations demonstrate that DDSA mutations promote opening of X-gate

The X-Gate remains closed in MD simulations of WT TASK-1 but opens with either N133S or L239P mutations.

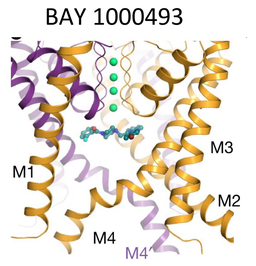


The hydrogen bond between N133 and A237 is crucial to stabilize the X-gate in a closed position.

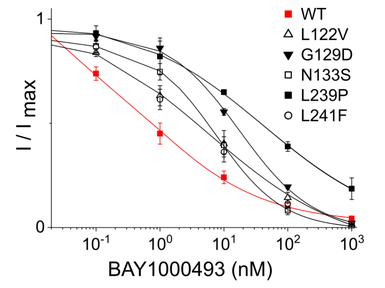




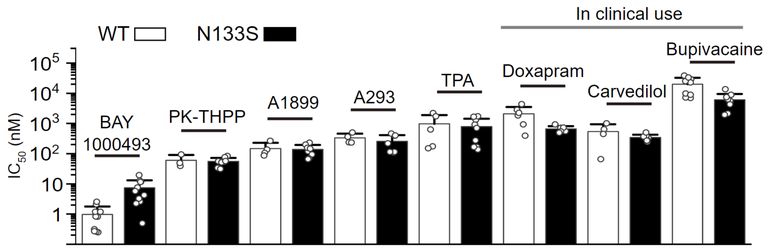
Pharmacology and regulation



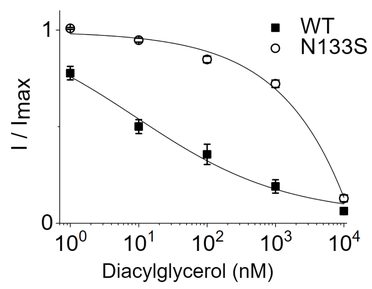
Rödström, et al. (*Nature* 2020)

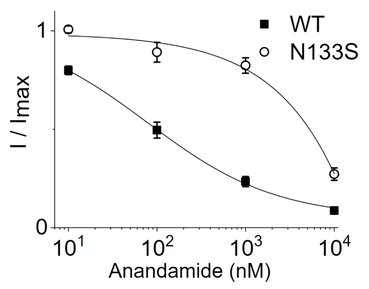


- BAY1000493 is an high affinity blocker of TASK-1. A similar drug is currently in clinical trials for treatment of sleep apnea.
- DDSA mutant channels retain ability to be inhibited by nanomolar concentrations of BAY1000493

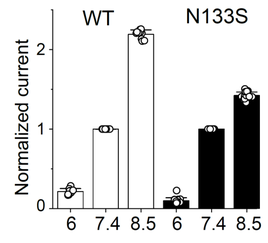


➢ The most common TASK-1 blockers also inhibit TASK-1 N133S.



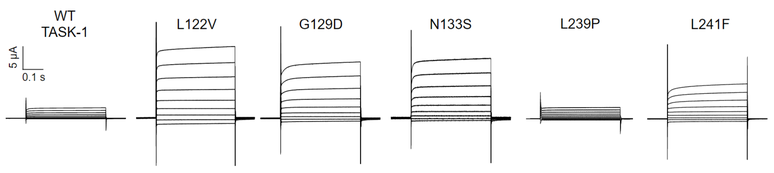


Inhibition by the physiological regulators Diacylglycerol and Anandamide is reduced in TASK-1 N133S compared to WT. But regulation by external pH mostly still intact.

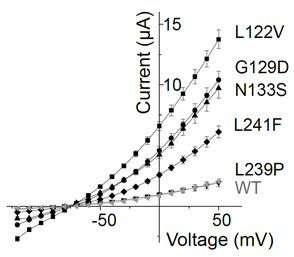


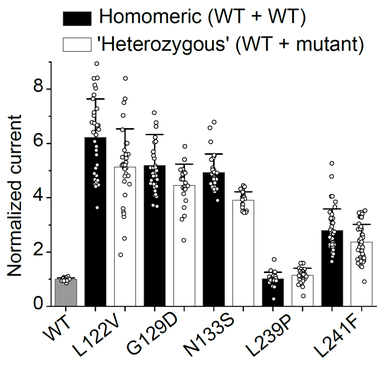
DDSA mutations cause a Gain-of-Function

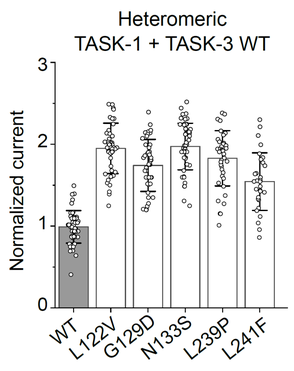
Two electrode voltage clamp (TEVC) whole cell recordings in *Xenopus* oocytes show increased currents compared to WT TASK-1 – the only exception is L239P.



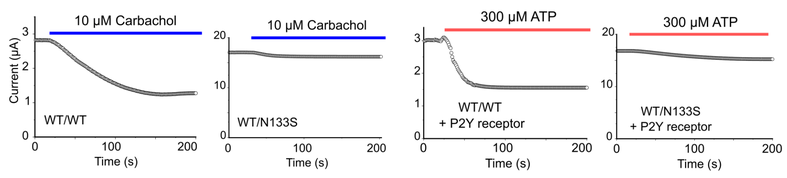
DDSA mutants also display increased currents when co-expressed with WT TASK-1 as heterozygous channels and co-expressed with WT TASK-3 as heteromeric channels.

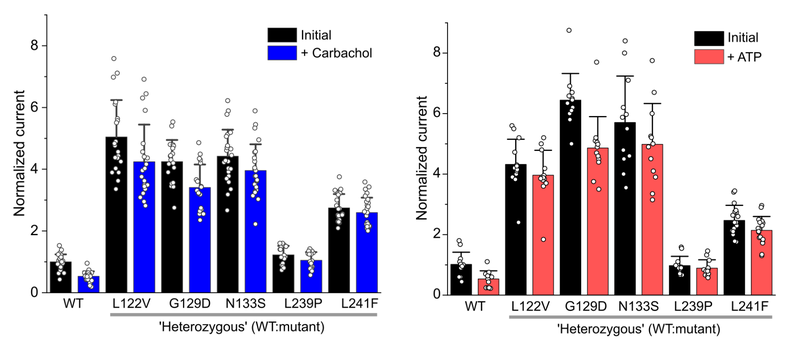






Reduced GPCR mediation





- DDSA mutant channels exhibit almost no GPCR-mediated inhibition either as homomeric or 'heterozygous' channels
- This amplifies the gain-of-function effect of DDSA mutants and also demonstrates a GoF for the L239P mutant with more residual current than WT after GPCR-mediated inhibition

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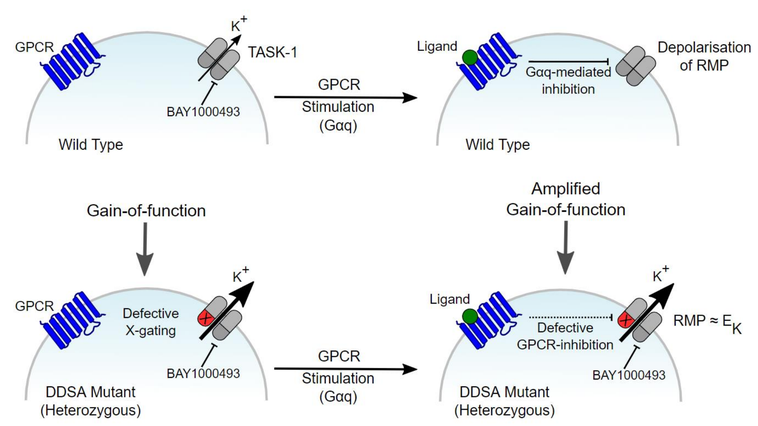
UKRI Biotechnology and Biological Sciences Research Council

DFG Deutsche Forschungsgemeinschaft

BAYER

Summary



- **Clear link between DDSA mutations and Developmental Delay with Sleep Apnea**
- **DDSA mutations are gain-of-function even as 'heterozygous' channels**
- **They have a higher open probability caused by a defective X-gating**
- **They are less sensitive to GPCR mediation**
- **DDSA mutations still retain sensitivity to inhibition by clinically relevant drugs e.g. BAY1000493. Similar drug used in KOALA trial (NCT04236440)**