Synaptic actions of FGF -1 in the arcuate nucleus of the hypothalamus and dorsal vagal complex

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Obesity

Obesity is a worldwide epidemic

Contributes to multiple top 10 global causes of death including:

- Heart disease
- Stroke
- Lower respiratory infections

Diabetes (~450 million people worldwide)

- Roughly 40% of adults in U.S. are obese
- 72% when including those overweight

Massachusetts 5th *least* obese state!

CDC 2017
WHO 2018
Flegal *JAMA* 2013
Ogden et al. 2014

Paucity of therapeutics

Conventional weight loss programs

- Less than 3% are at or below post-treatment weight 4-5 years after a successful weight-loss program

Bariatric Surgery

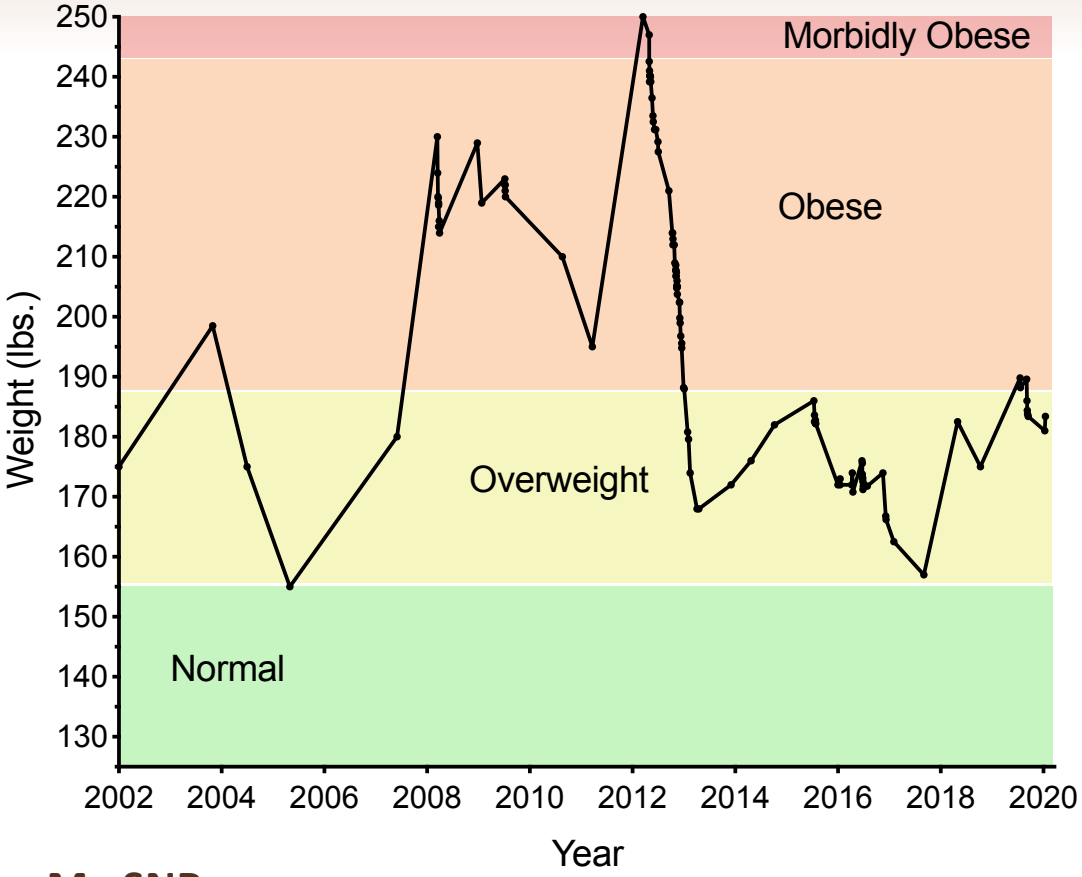
- Most effective
- Highly invasive
- Paired with conventional weight loss programs

Current Pharmaceuticals

- Adverse side-effects
- Low compliance rate
- Few effective options

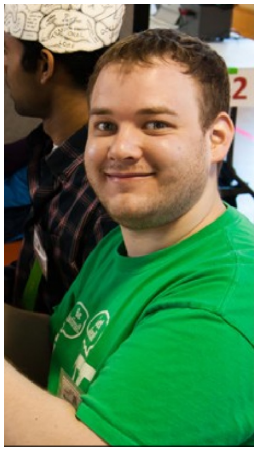
Kramer 1989
Powell 2011
Service 2013
Wing R 2005

“It’s easy- exercise and eat right.”

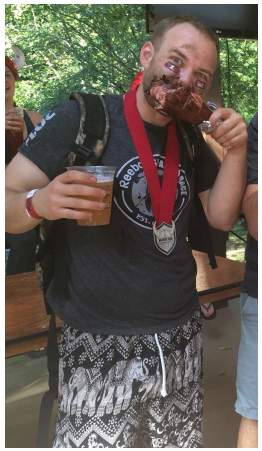


~35 mi/week

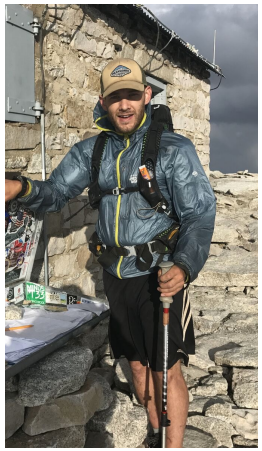
Sedentary



~35 mi/week plus strength



~10 mi/week



50-150 mi/week, plus strength, plus two races a month

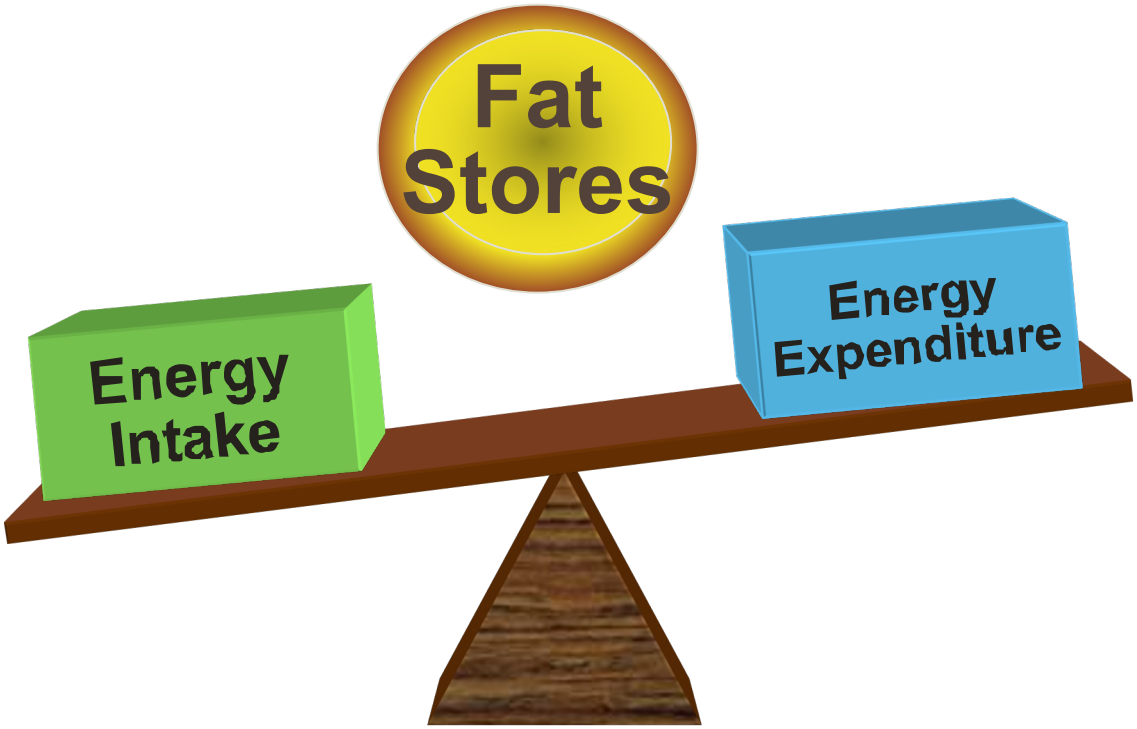
My SNPs

Rs1121980 (C;T) – 1.67 higher risk
Rs17817449(G;T) – 1.3x higher risk
Rs5746059(A;G) – 4.6% higher fat mass
Rs12970134(A;G) – **MC4R gene variant**

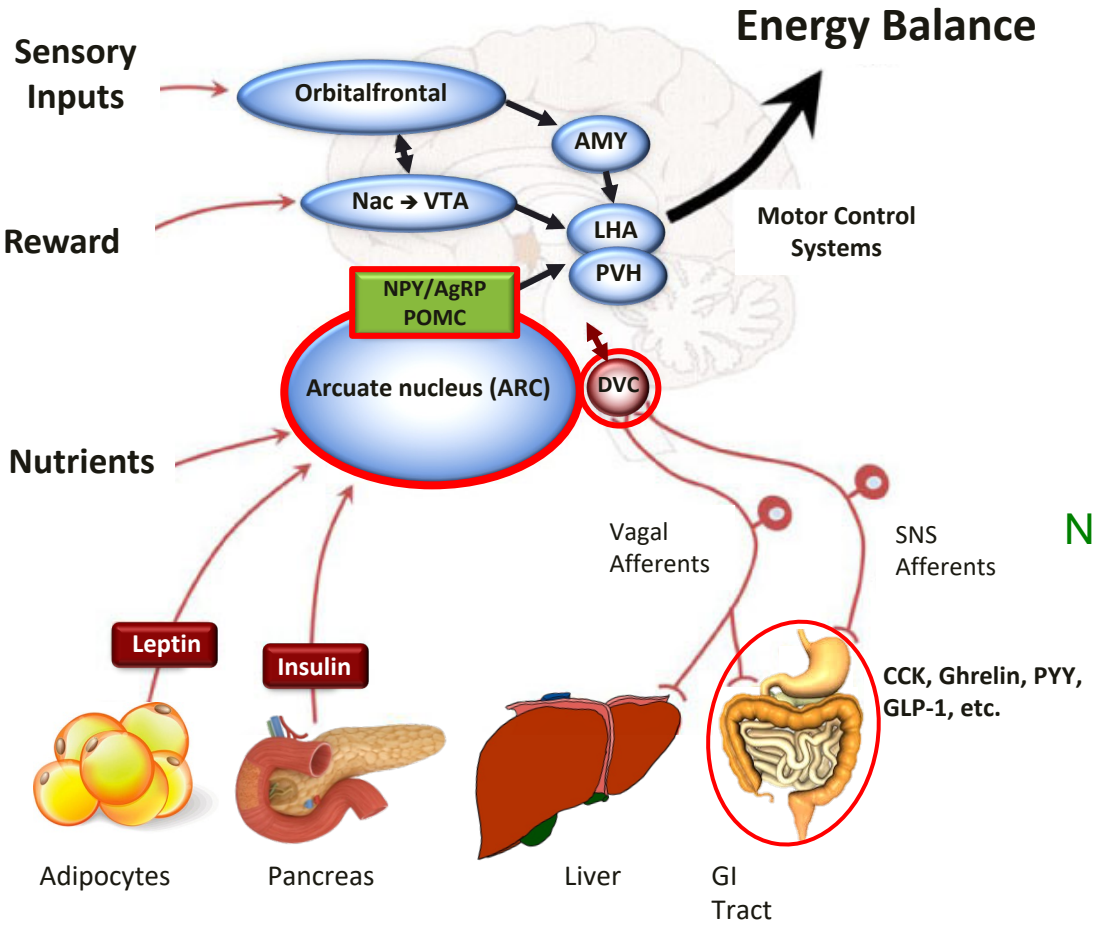
Current attempts are not an effective long-term strategy

What causes obesity?

Long-term positive energy balance



What regulates food intake and energy metabolism?



Orexigenic

(↑ Food intake)

- NPY
- AgRP
- Ghrelin
- Insulin

Anorexigenic

(↓ Food intake)

- αMSH
- Leptin
- PYY
- GLP-1
- CCK
- Insulin

NPY/AgRP neurons ⊣ (GABA) POMC neurons

Primary central circuits:

- Dorsal vagal complex (DVC)
- Hypothalamus

Cui H 2005
Hazell T 2015
Hyun-Ju Kim 2011
Koch M 2014
Tschop M 2001

Recent Projects

- Impact of postnatal overnutrition on neural development and central leptin signaling
- Drug discovery partnership
- Industry-academic collaboration to investigate the role of reelin in metabolic systems
- Central actions of fibroblast growth factor -1 (FGF1)

Fibroblast Growth Factor -1 (FGF1)

Member of FGF protein family (~17 kDa)

Involved in:

- Embryonic development
- Cell growth
- Tissue repair

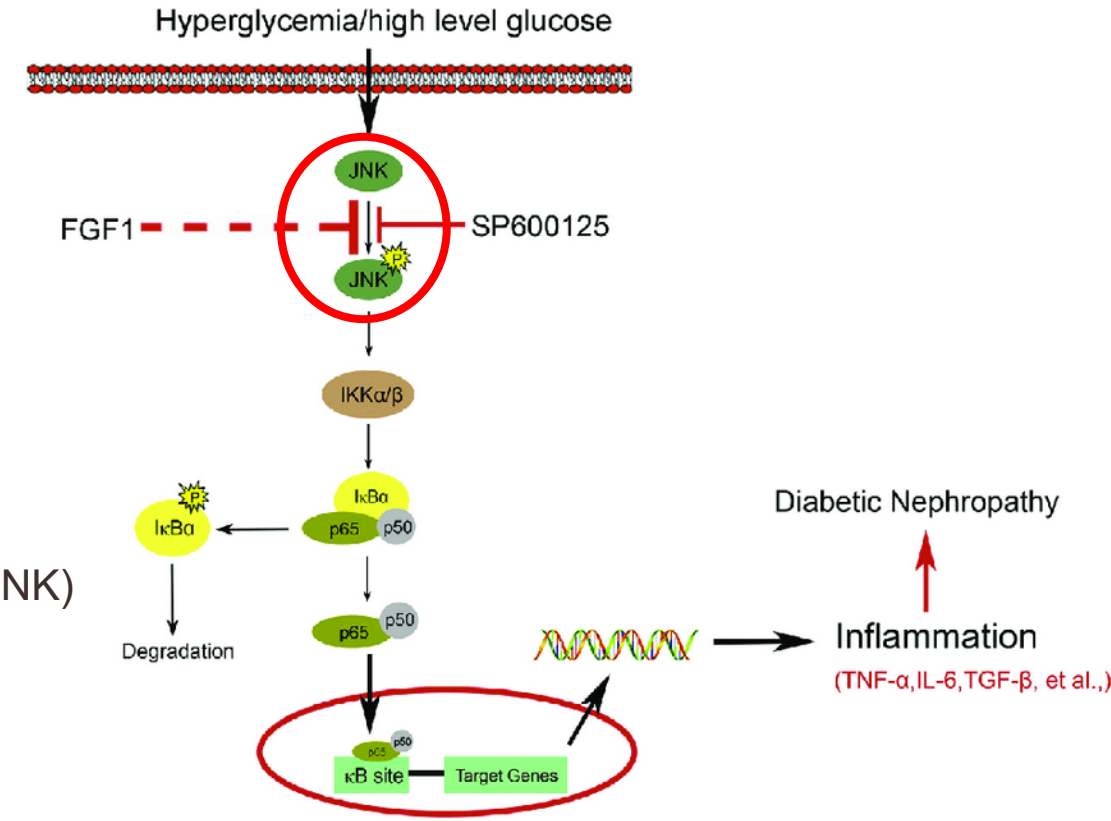
Receptor binding

- Tyrosine kinase receptors

Intracellular (c-jun N-terminal kinase; JNK)

Ubiquitous expression

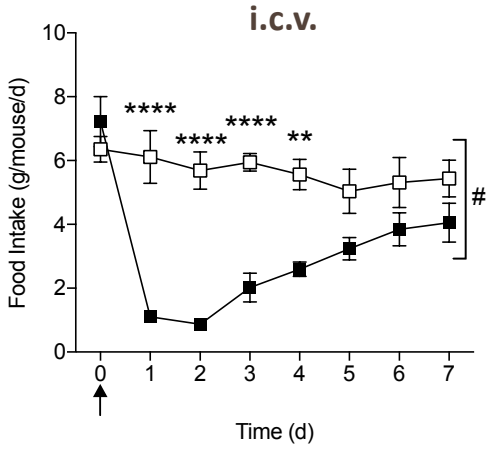
Knockouts are highly diabetic on HFD



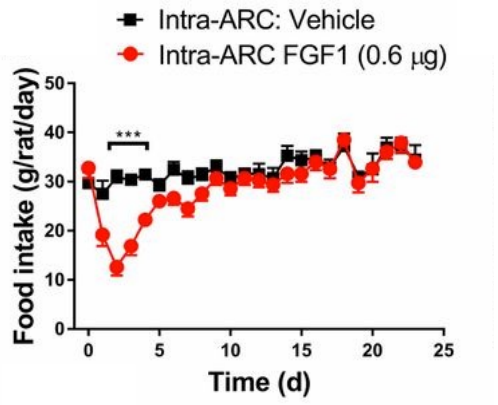
Jonker JW, *Nature* 2012
Ornitz DM, *Rev Dev Bio* 2015
Liang G, *Kidney Int.* 2017

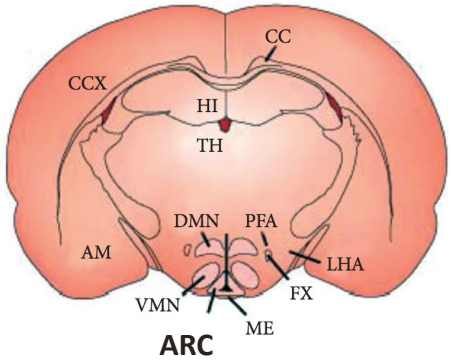
FGF1 reduces food intake in diabetic rodent models

db/*db* mouse model



Zucker diabetic rat (ZDF)



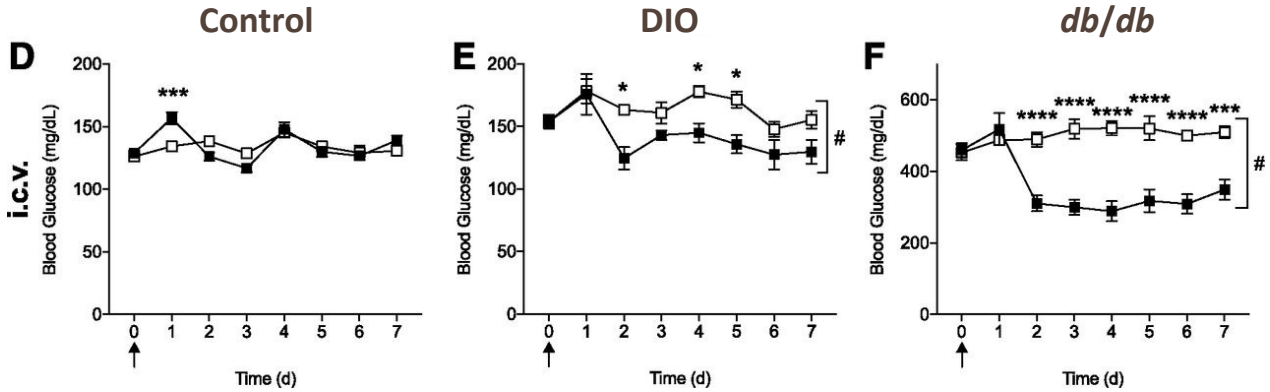


db*/*db – leptin receptor mutation
ZDF – leptin receptor mutation

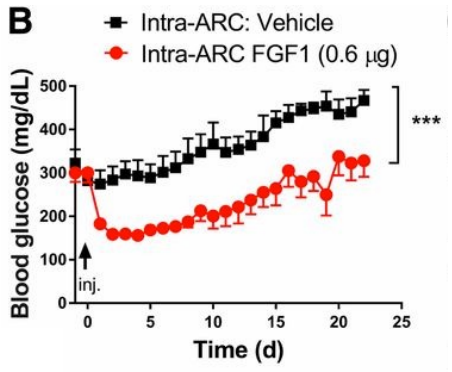
Tennant K, *Diabetes* 2019
Brown M, *Diabetes* 2019

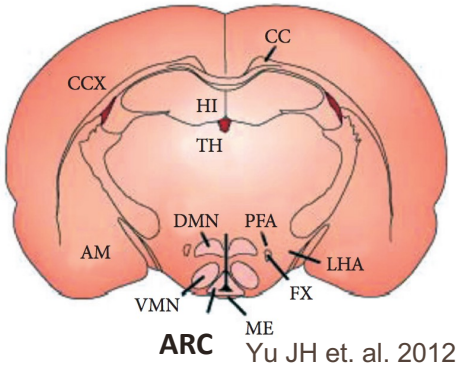
FGF1 reduces blood glucose in DIO and diabetic mouse models

Mouse Models



Zucker Diabetic Rat (ZDF)





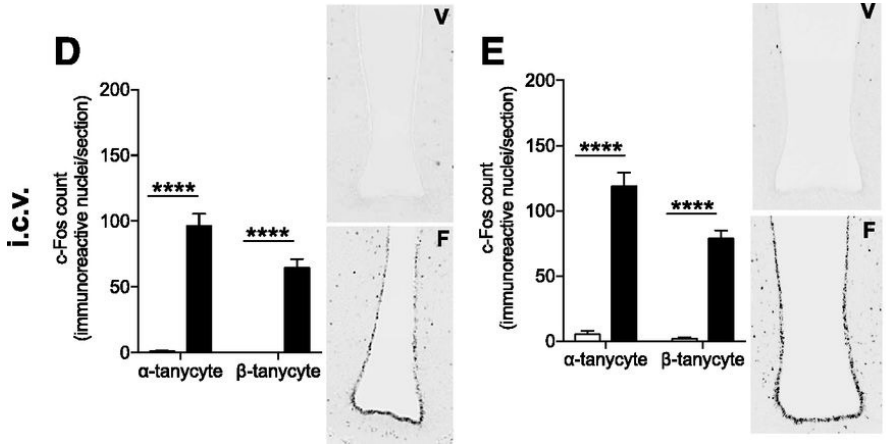
Yu JH et. al. 2012

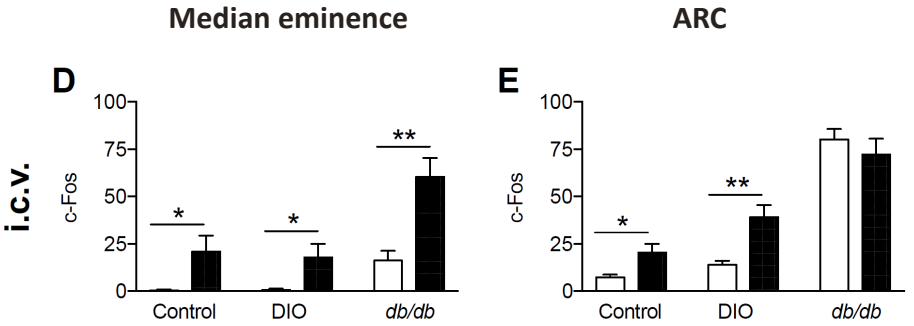
DIO – diet induced obese
db/db – leptin receptor mutation
ZDF – leptin receptor mutation

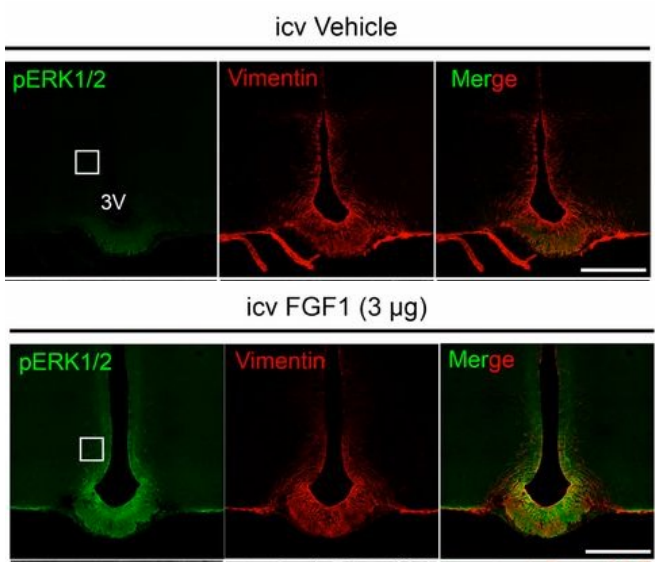
Tennant K, *Diabetes* 2019
Brown M, *Diabetes* 2019

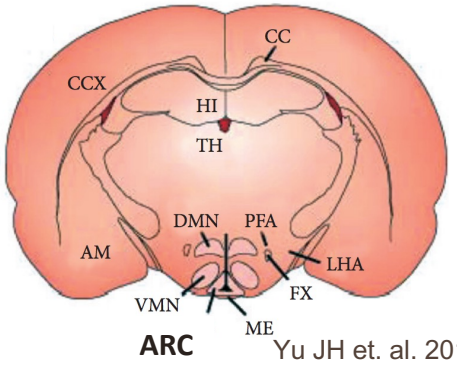
FGF1 increases cFos and pERK1/2 in the ARC, median eminence, and in tanycytes

Control DIO





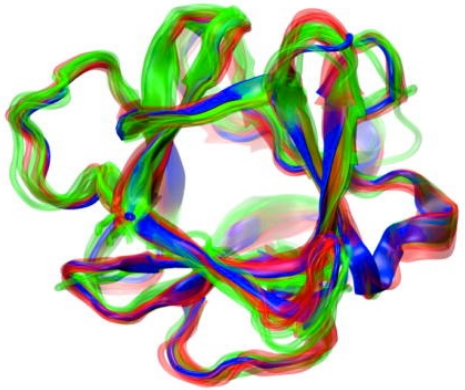




Yu JH et. al. 2012

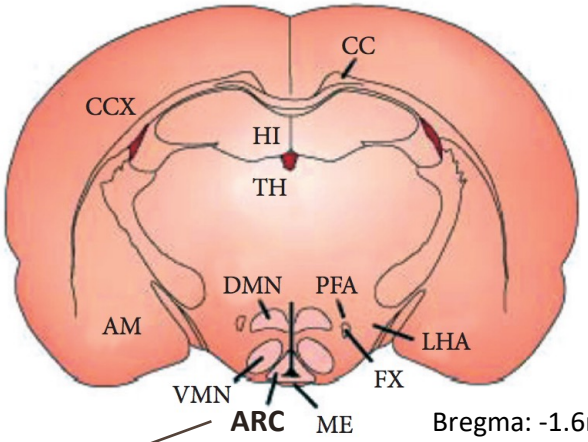
Tennant K et. al. 2019
Brown M et. al. 2019

Does FGF1 act directly on ARC-POMC or –NPY neurons and, if so, what are the potential mechanisms by which it acts?



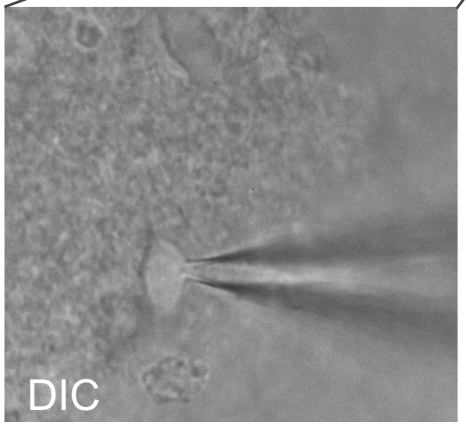
Methodology

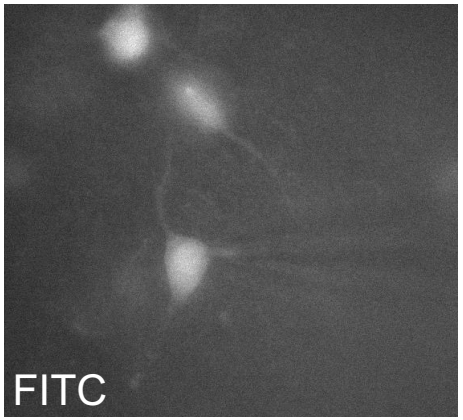
- **Coronal hypothalamic brain slice**
  - Preserves the arcuate nucleus
- **Patch-clamp in the arcuate**
  - Measure changes in currents and voltages
- **Transgenic mice**
  - Proopiomelanocortin (POMC) -EGFP
  - Neuropeptide Y (NPY) -GFP



Bregma: -1.6mm

Yu JH et. al. 2012

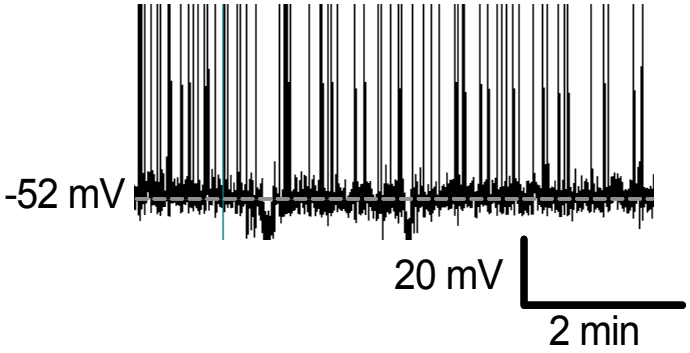




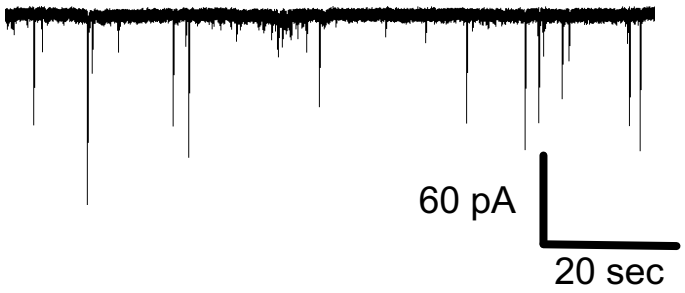
Methodology

Patch-clamp techniques:

- **Neuronal activity (Current Clamp)**
  - Membrane potential (mV)
  - Action potentials (Frequency (Hz))



- **Neurotransmitter release/receptor binding (Voltage Clamp)**
  - Voltage held at -60 mV
  - Measure changes in current:
    - Frequency (Hz)
    - Amplitude (pA)

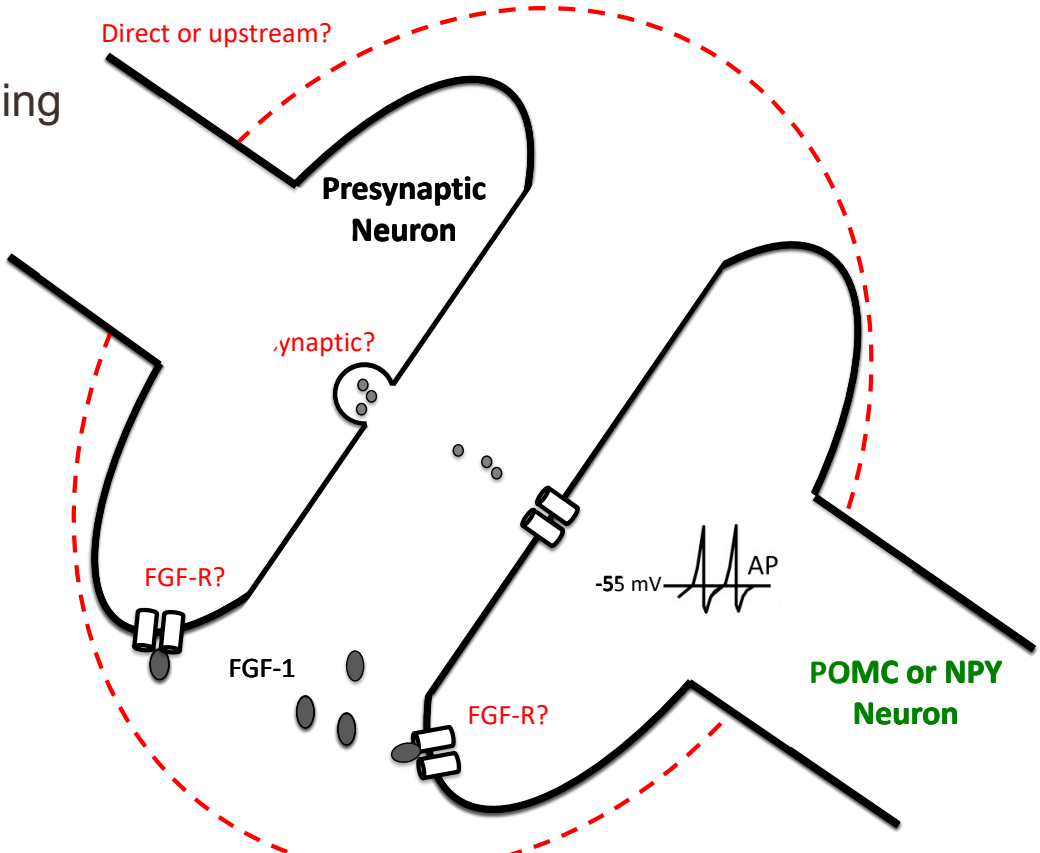


Objectives

1) Does FGF1 alter the activity of ARC-POMC or -NPY neurons?

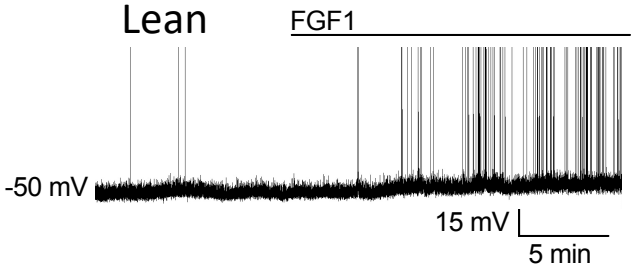
2) Does FGF1 alter neurotransmitter release?

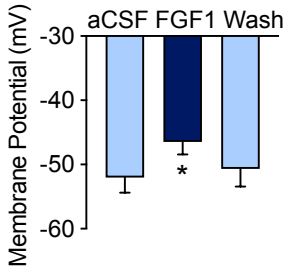
3) What receptor mediates FGF1 signaling on these neurons?

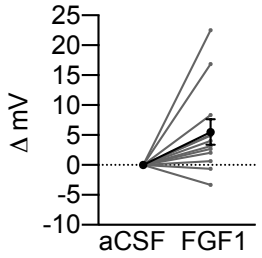


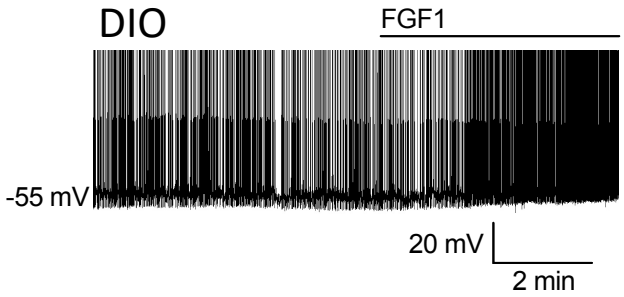
FGF1 activates ARC-POMC-EGFP neurons

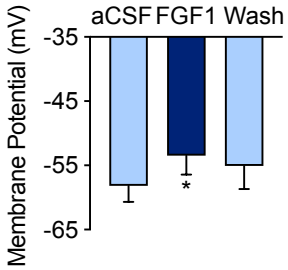
POMC-EGFP



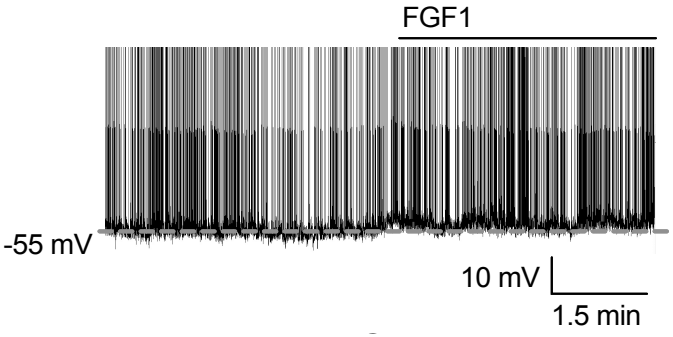


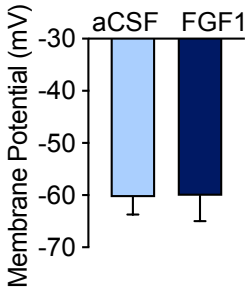


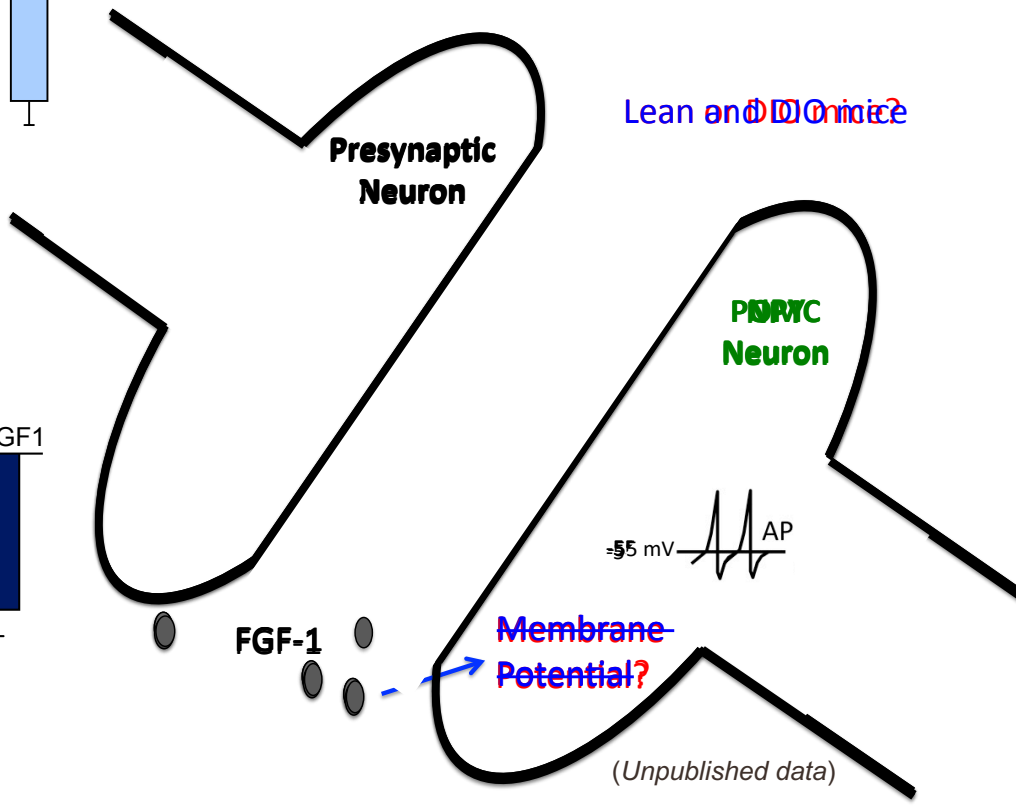




NPY-GFP



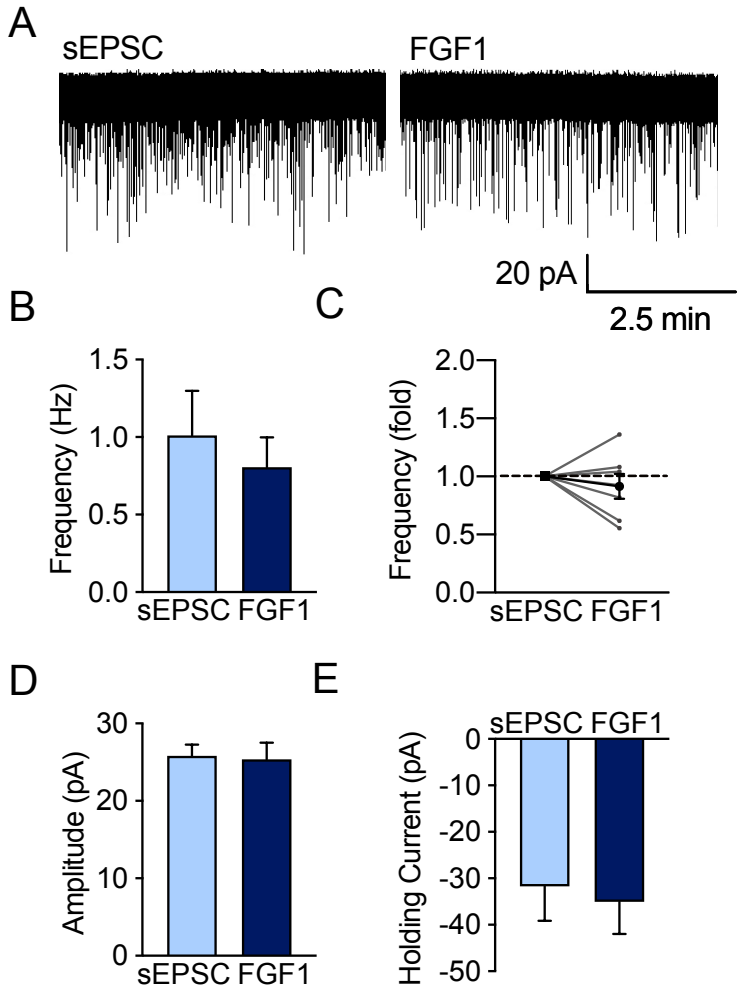


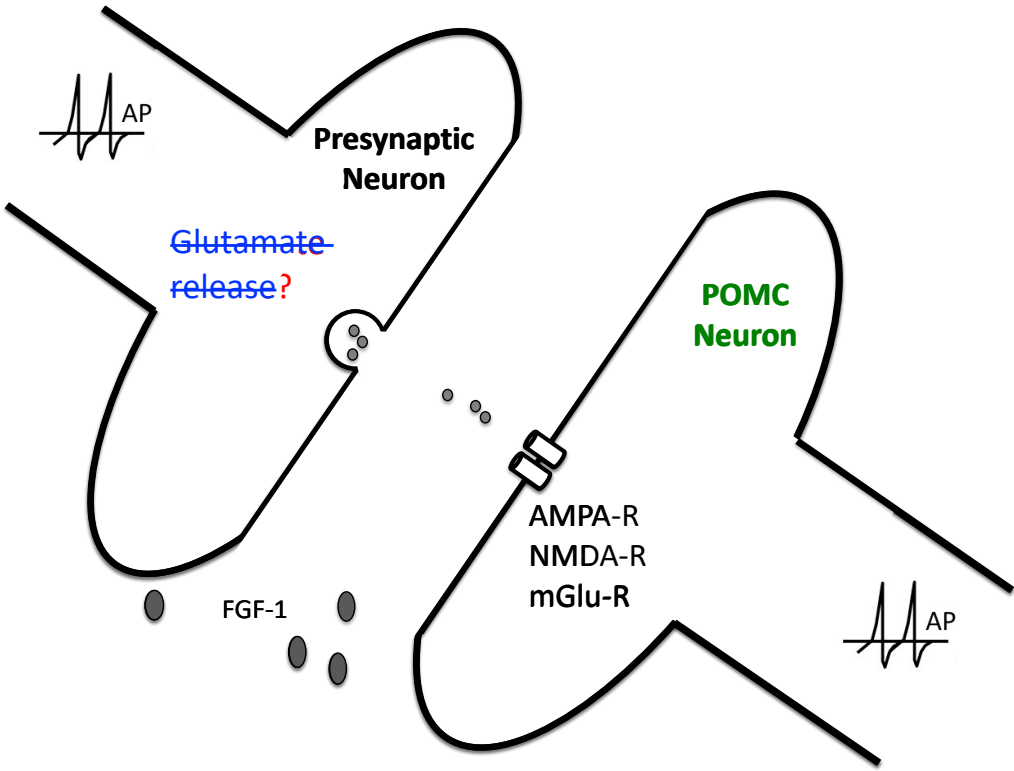


FGF1 does not alter spontaneous excitatory inputs on ARC-POMC neurons

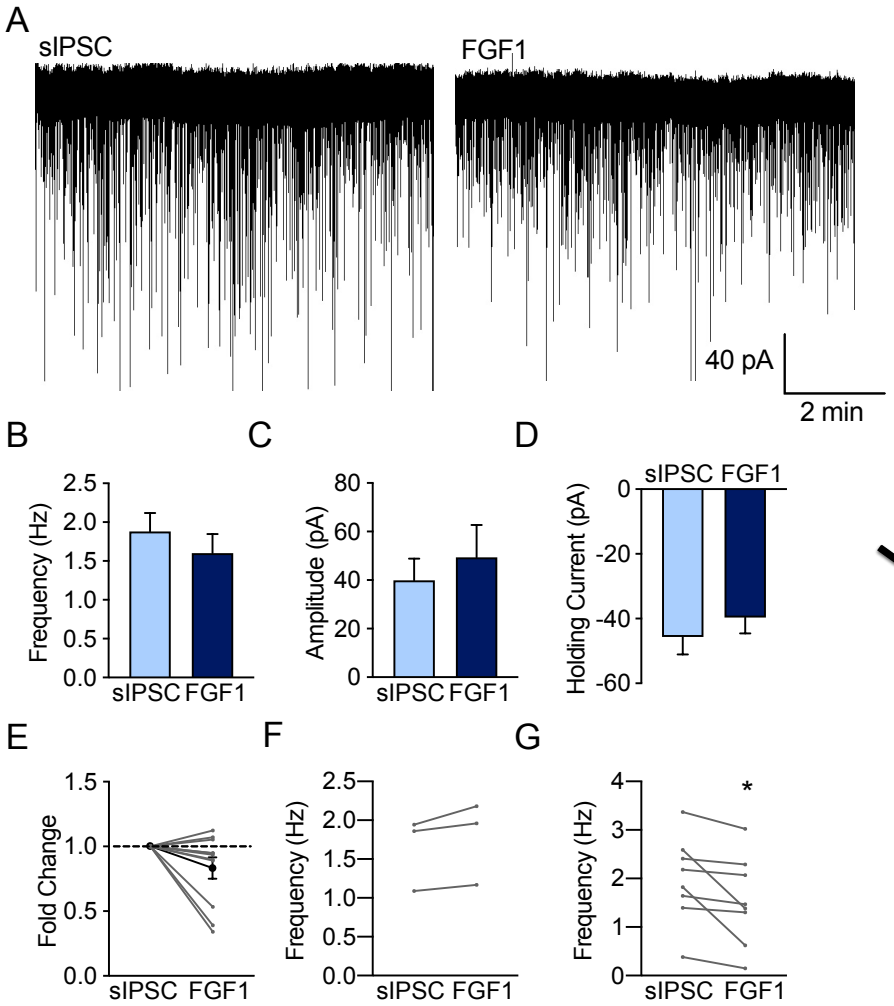
Excitatory postsynaptic currents (EPSCs)

Bicuculline (BIC) - $GABA_A$ receptor antagonist



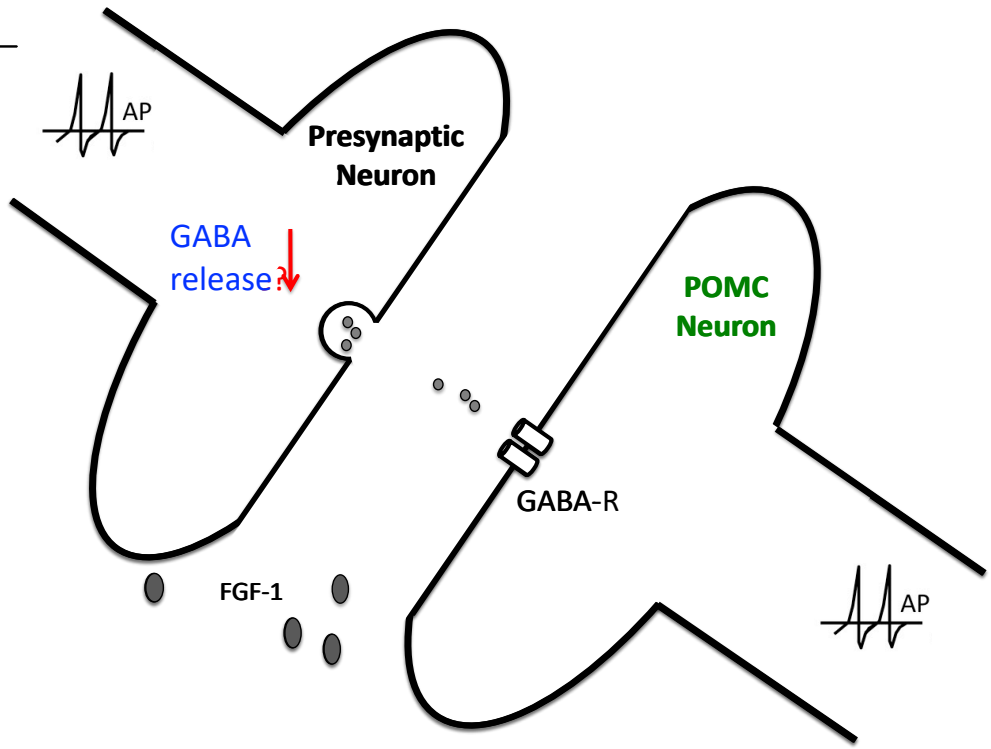


FGF1 decreases spontaneous inhibitory inputs on most ARC-POMC neurons



Inhibitory postsynaptic currents (IPSCs)

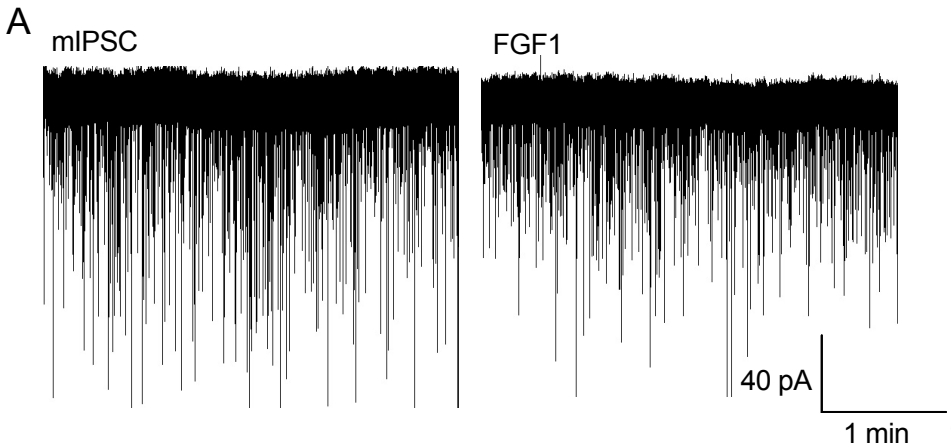
APV; NMDA receptor antagonist
CNQX; AMPA receptor antagonist

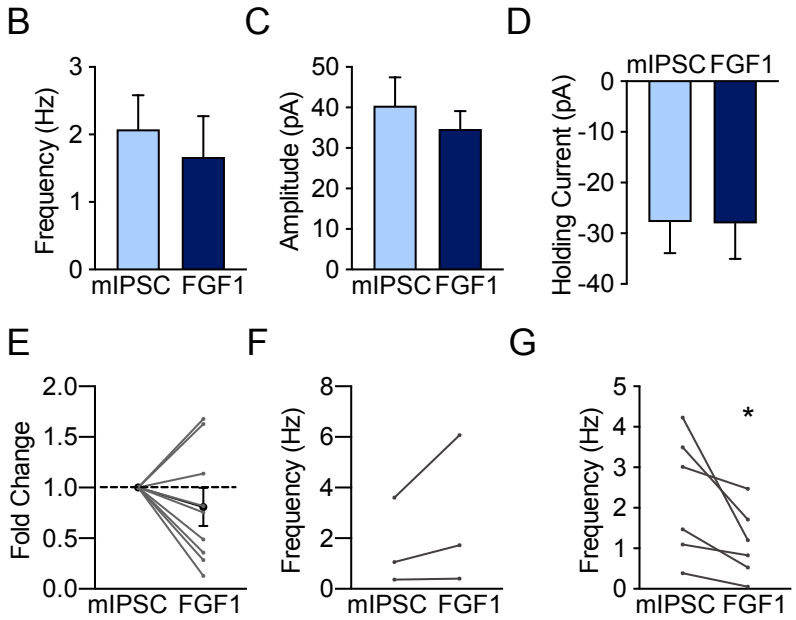


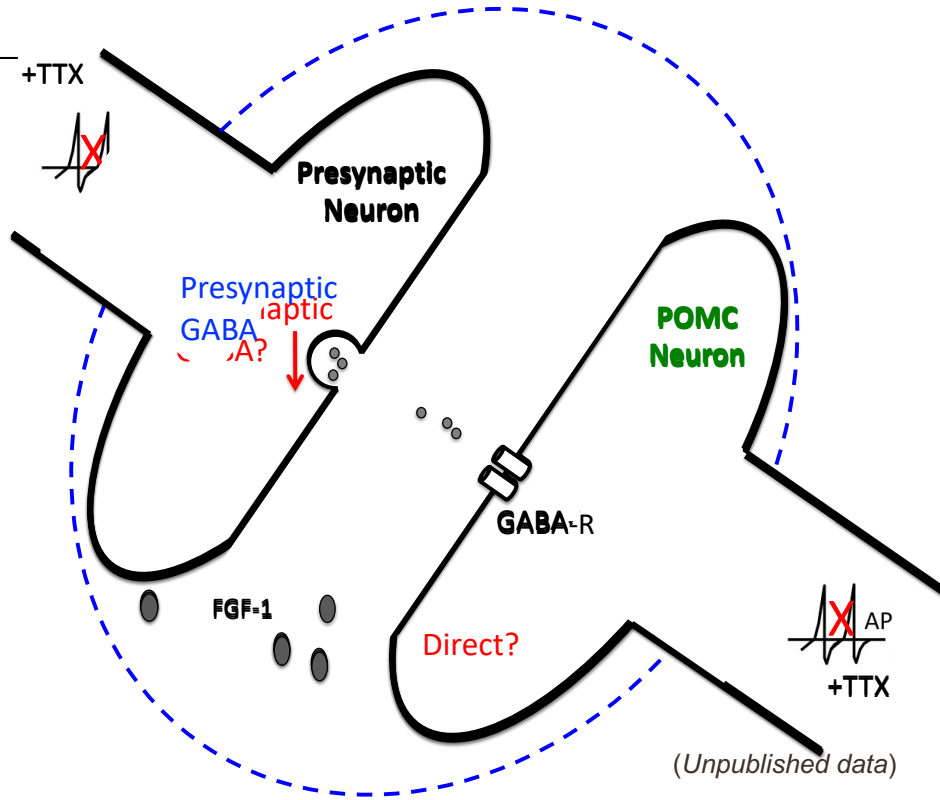
(*Unpublished data*)

FGF1 locally decreases inhibitory inputs on most ARC-POMC-EGFP neurons

Tetrodotoxin (TTX); Na^+ channel blocker
APV; NMDA receptor antagonist
CNQX; AMPA receptor antagonist



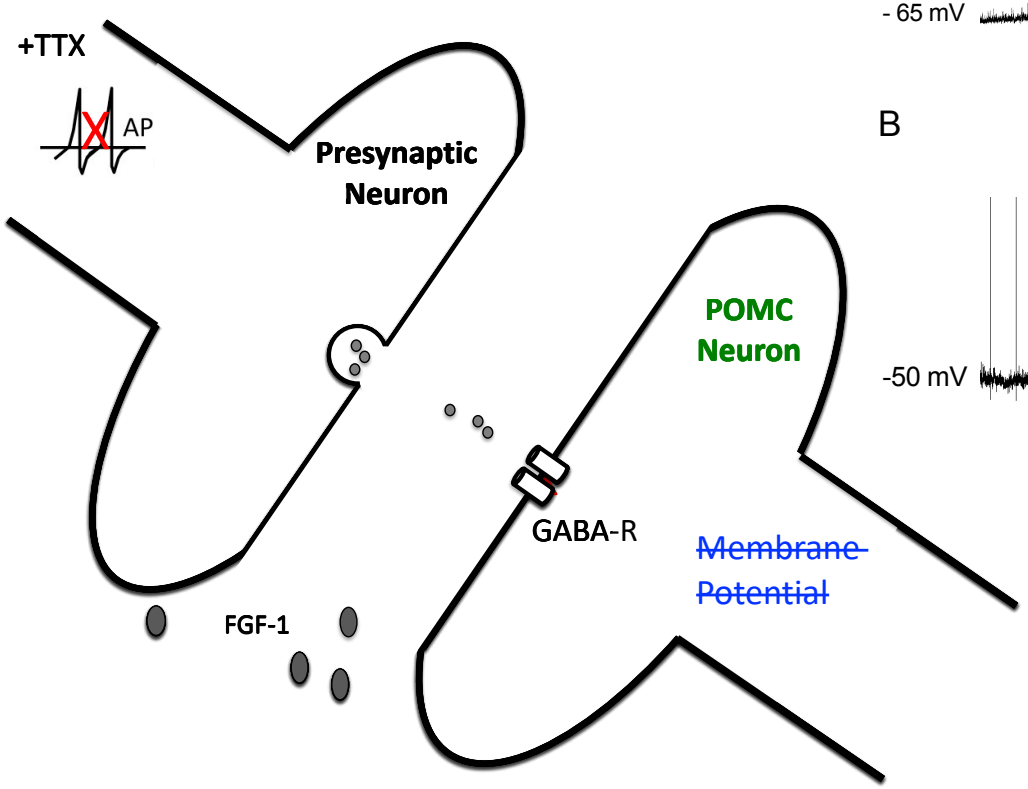


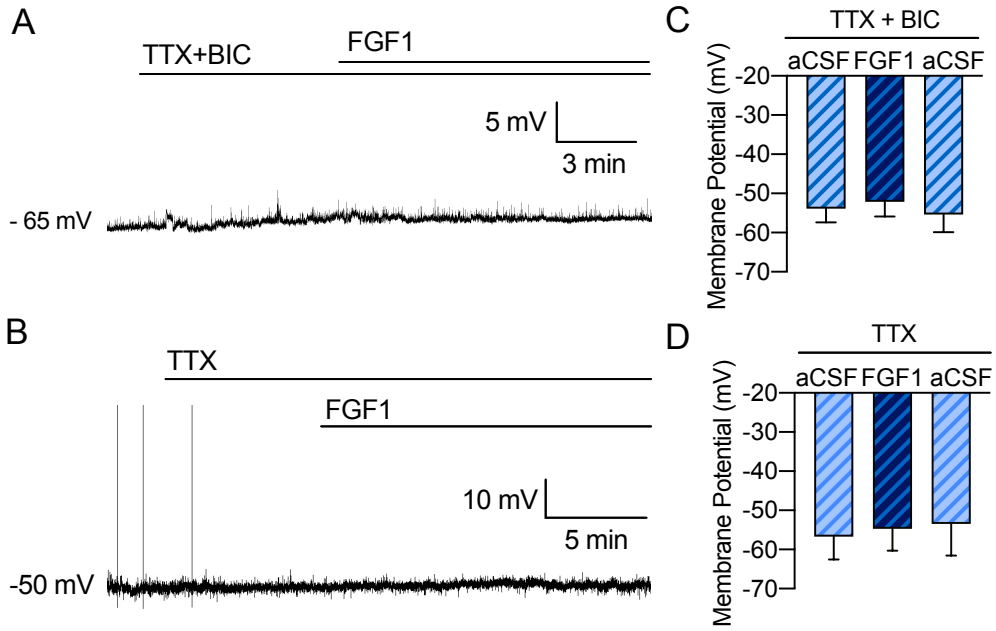


(*Unpublished data*)

FGF1 activation of ARC-POMC-EGFP neurons is indirect

Tetrodotoxin (TTX) – Na^+ channel blocker
Bicuculline (BIC) - $GABA_A$ receptor antagonist

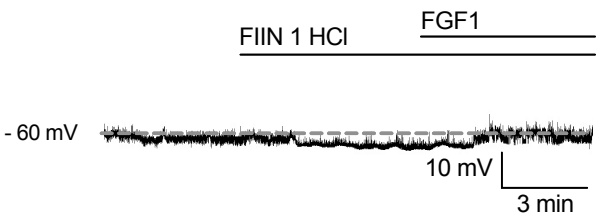




(*Unpublished data*)

FGF1 actions on ARC-POMC neurons are independent of FGF receptors

C FGFR 1, 2, 3, 4 inhibitor



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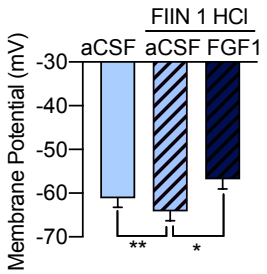
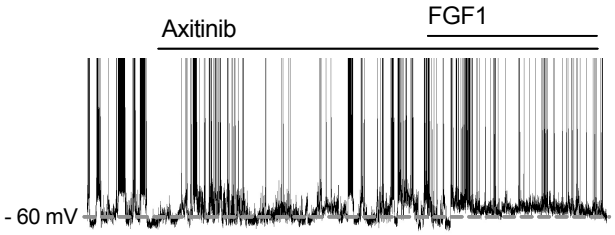


VEGFR 1, 2, 3, 4 inhibitor

E



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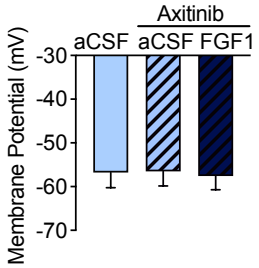
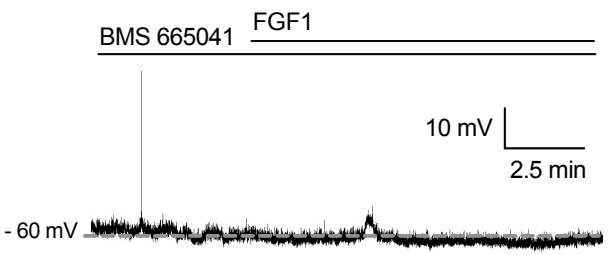


VEGFR 1, 2 inhibitor

G



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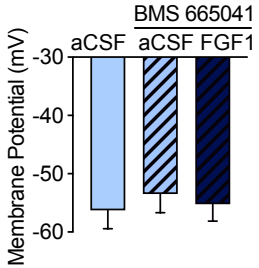
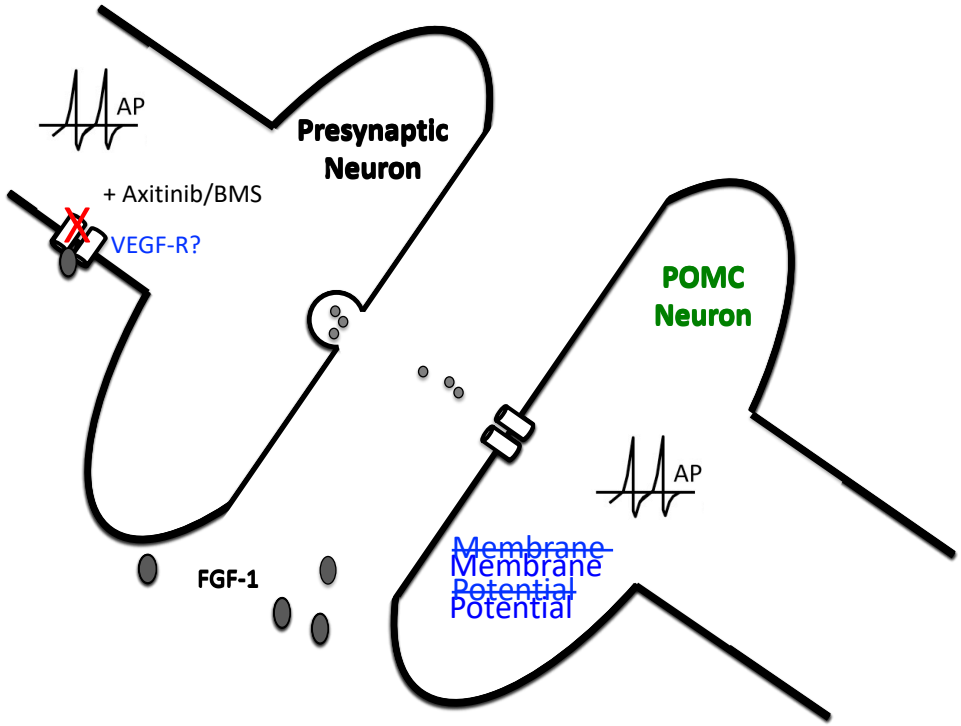


VEGFR

- Regulates tanycyte permeability (leptin transport)
- Tyrosine kinase with similar binding domains

- FGF impacts VEGF signaling and VEGFR expression
- FGF1 most promiscuous FGF



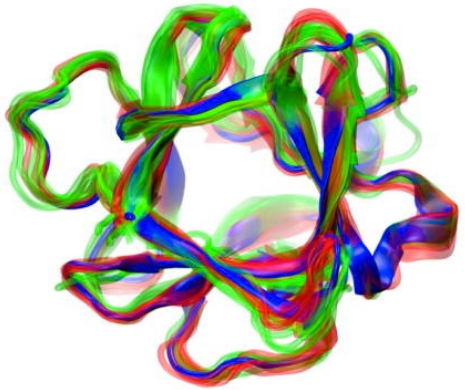
(*Unpublished data*)

Summary

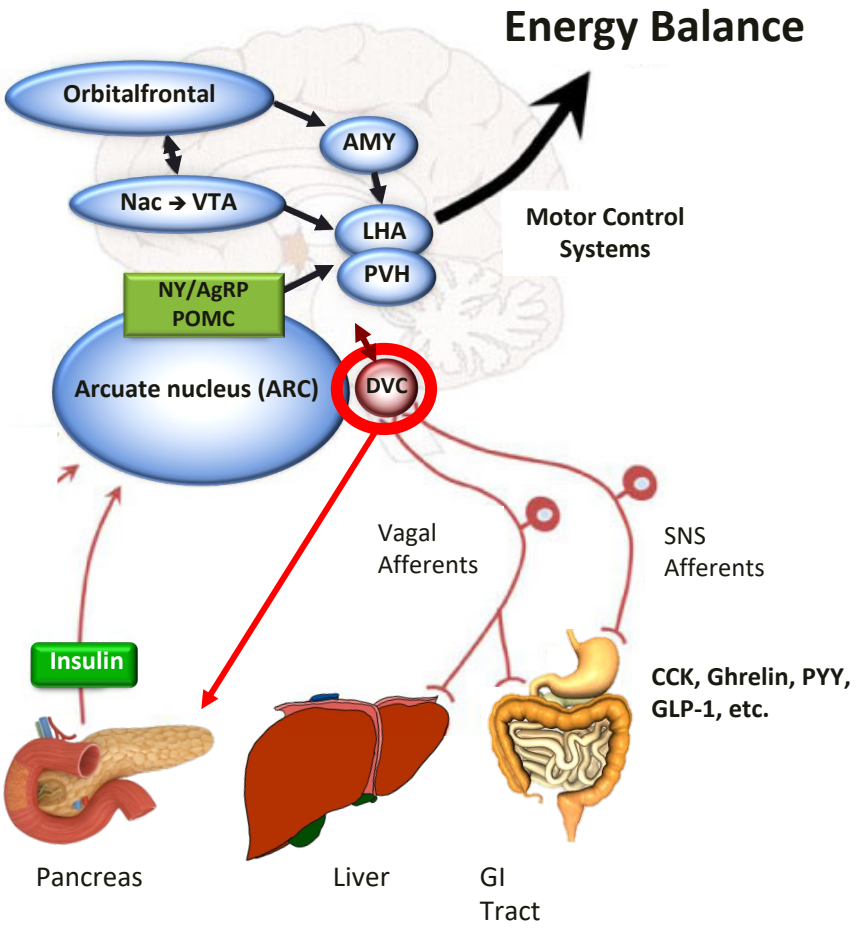
In the arcuate nucleus:

- FGF1 depolarized ARC-POMC, but not NPY neurons
- FGF1 decreases inhibitory inputs onto ARC-POMC neurons
- FGF1 activation of ARC-POMC neurons is indirect
- VEGF receptors are involved in FGF1 activation of ARC-POMC neurons

Does FGF1 have any actions in the hindbrain?

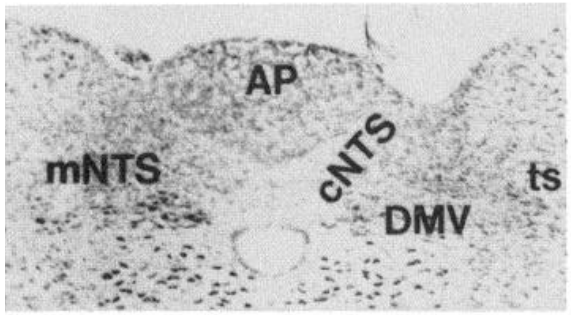


Role of dorsal vagal complex (DVC) in energy homeostasis



Dorsal vagal complex (DVC)

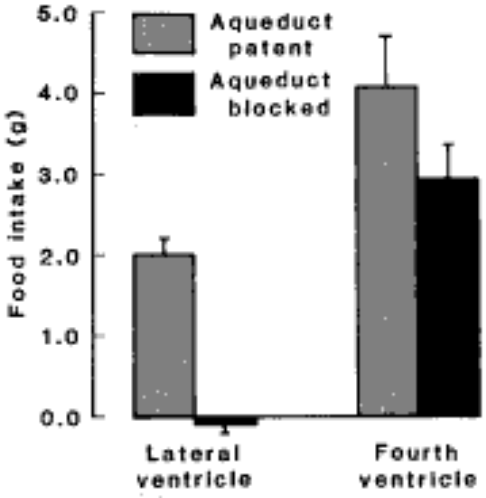
- Nucleus of the solitary tract (NTS)
- Area postrema (AP)
- Dorsal motor nucleus of the vagus (DMV)

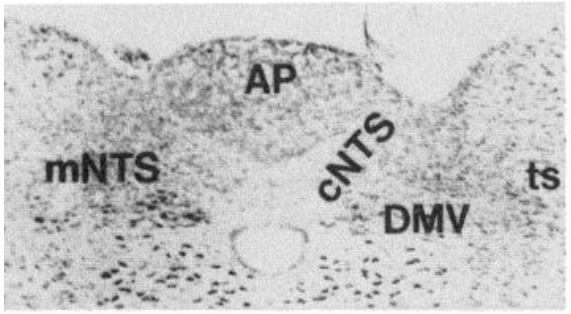


Ritter S, *AJP* 1990

Dorsal vagal complex (DVC) controls glucoprivic feeding

Glucoreceptors Controlling Feeding and Blood Glucose: Location in the Hindbrain





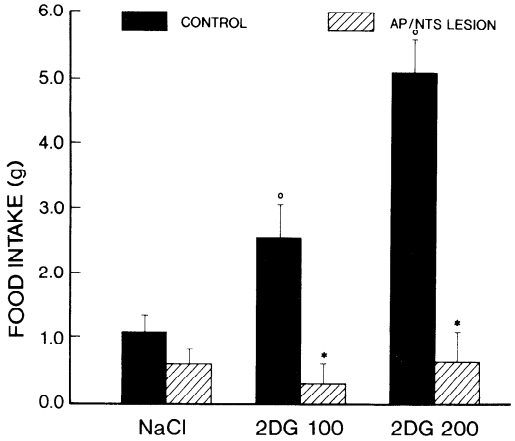
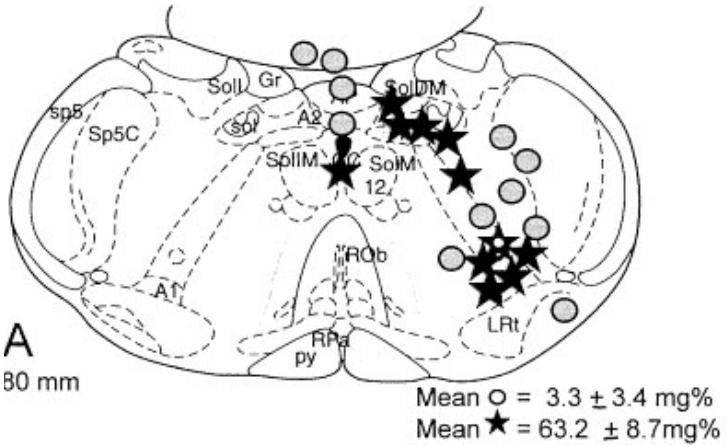
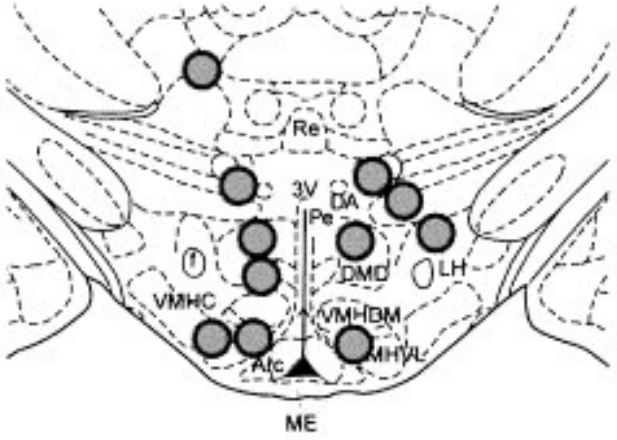


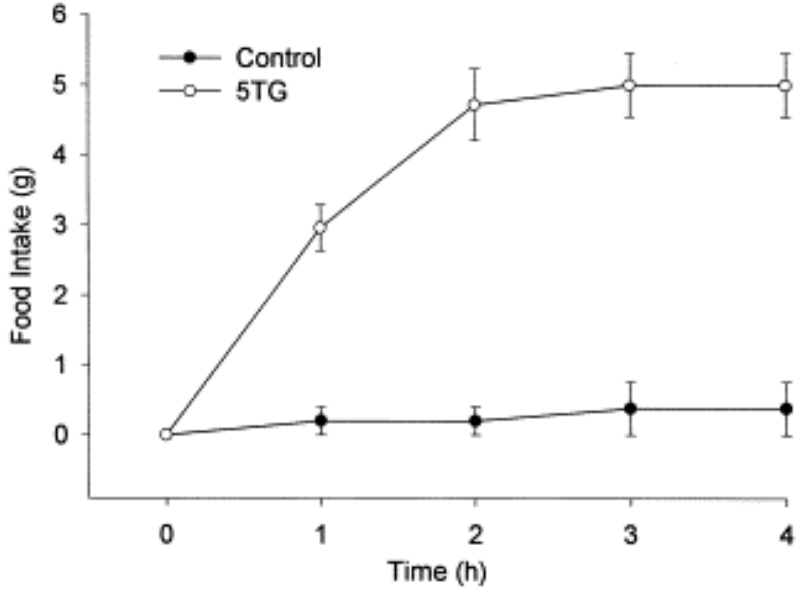
FIG. 6. Food intake of area postrema-nucleus of solitary tract (AP-NTS)-lesioned rats and controls during 6-h tests after subcutaneous injection of 2-deoxy-D-glucose (2-DG, 100 and 200 mg/kg) and NaCl (0.9%, mean of 4 tests). Rats were maintained and tested on medium-fat, high-carbohydrate powdered diet. * $P < 0.001$, lesion vs. control for same 2-DG dose; ° $P < 0.004$, control 2-DG vs. NaCl.

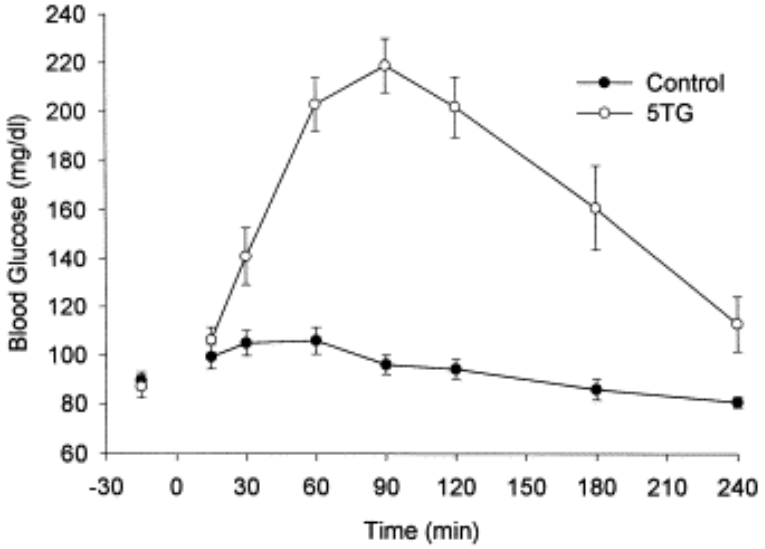
Ritter RC, *Science* 1981
Ritter S, *AJP* 1990

Dorsal vagal complex (DVC) controls feeding and blood glucose





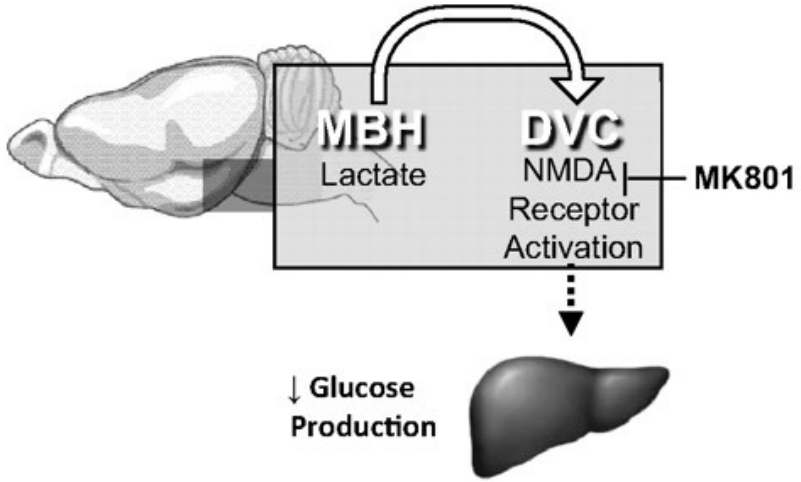


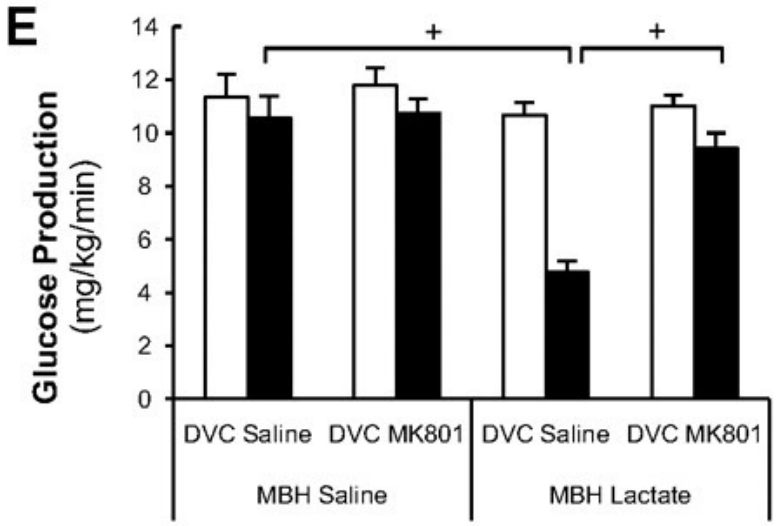


Ritter S, *Brain Res.* 2000

Dorsal vagal complex (DVC) controls ARC nutrient sensing

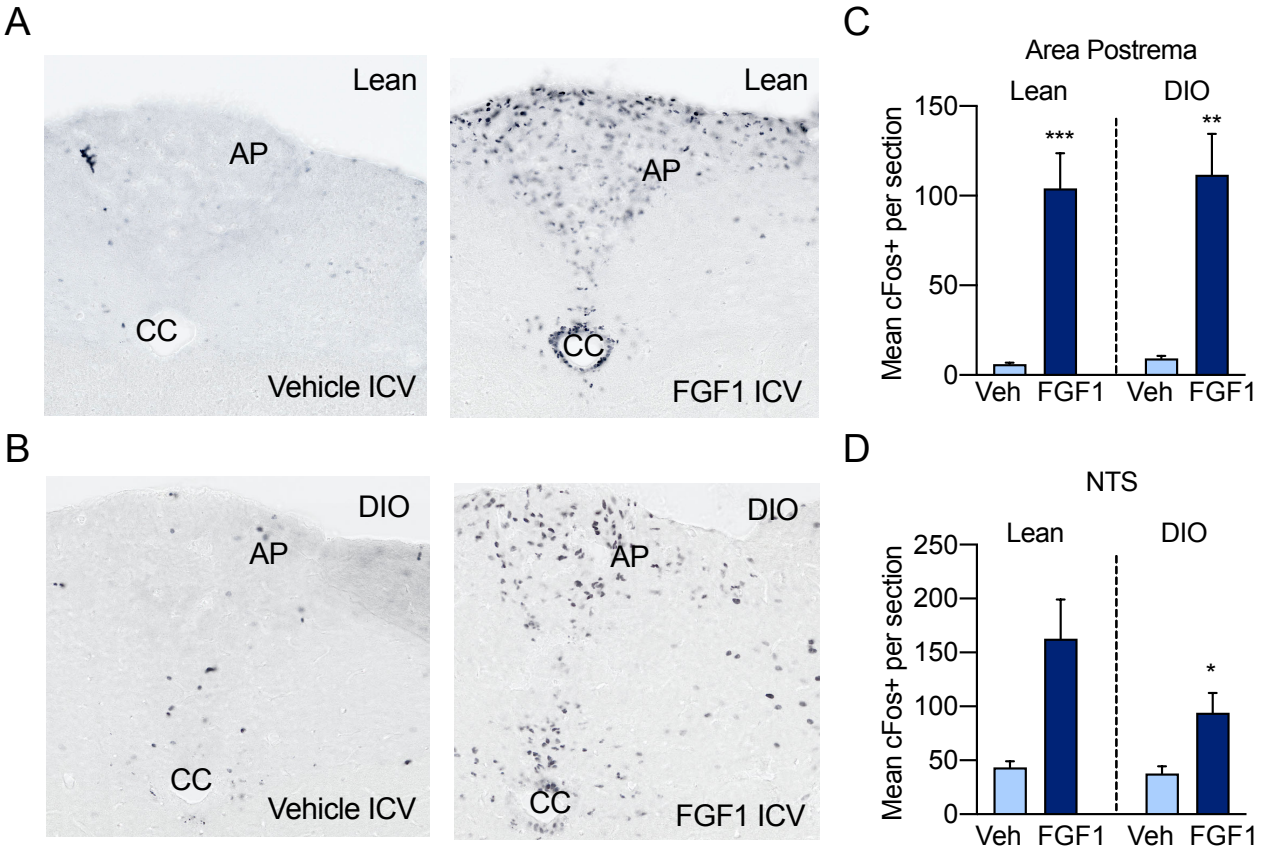
- Lactate infusion into medial basal hypothalamus (MBH) decreases hepatic glucose production
- NMDA receptor antagonist in DVC blocks this effect
- Same result for dominant negative AMPK adenovirus injections into MBH





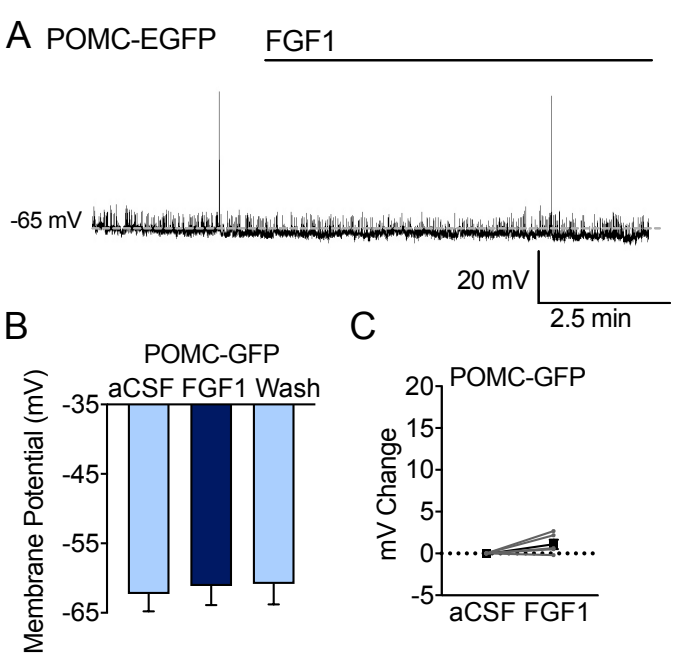
Lam CK, *Diabetes* 2011

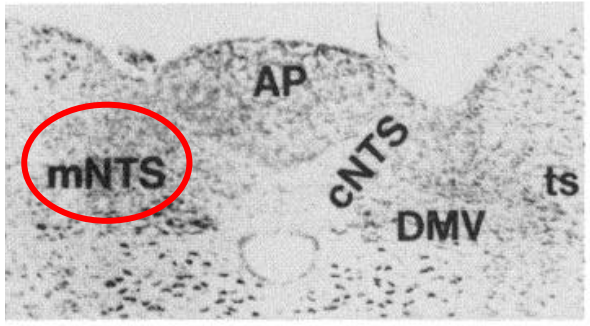
ICV FGF1 injection induces cFos in the DVC

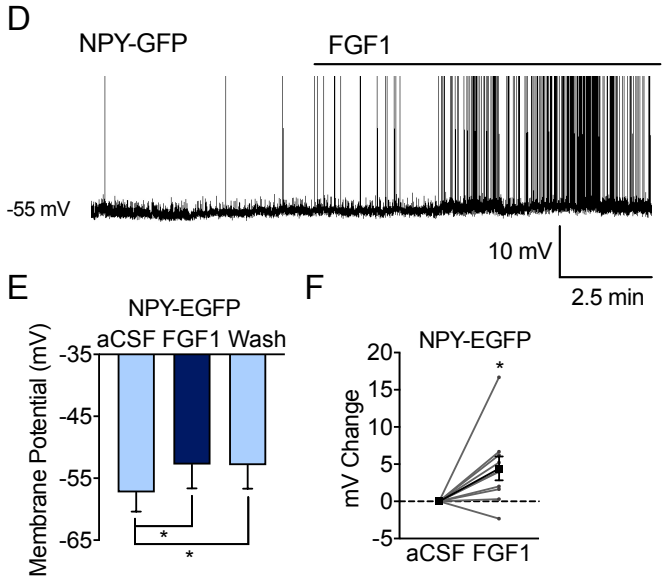


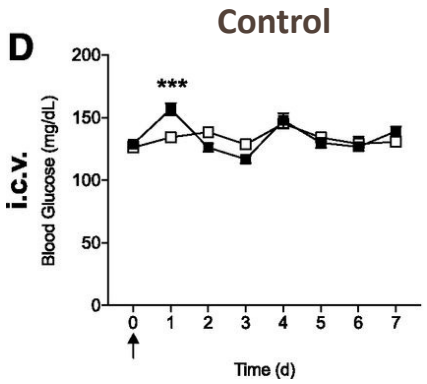
(*Unpublished data*)

FGF1 depolarizes NTS-NPY-GFP neurons





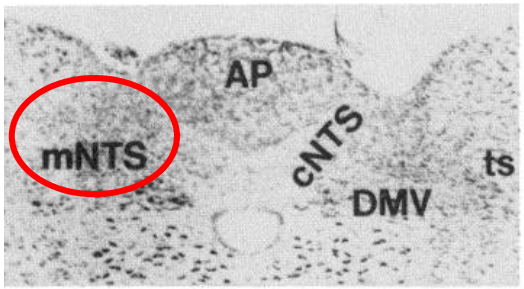


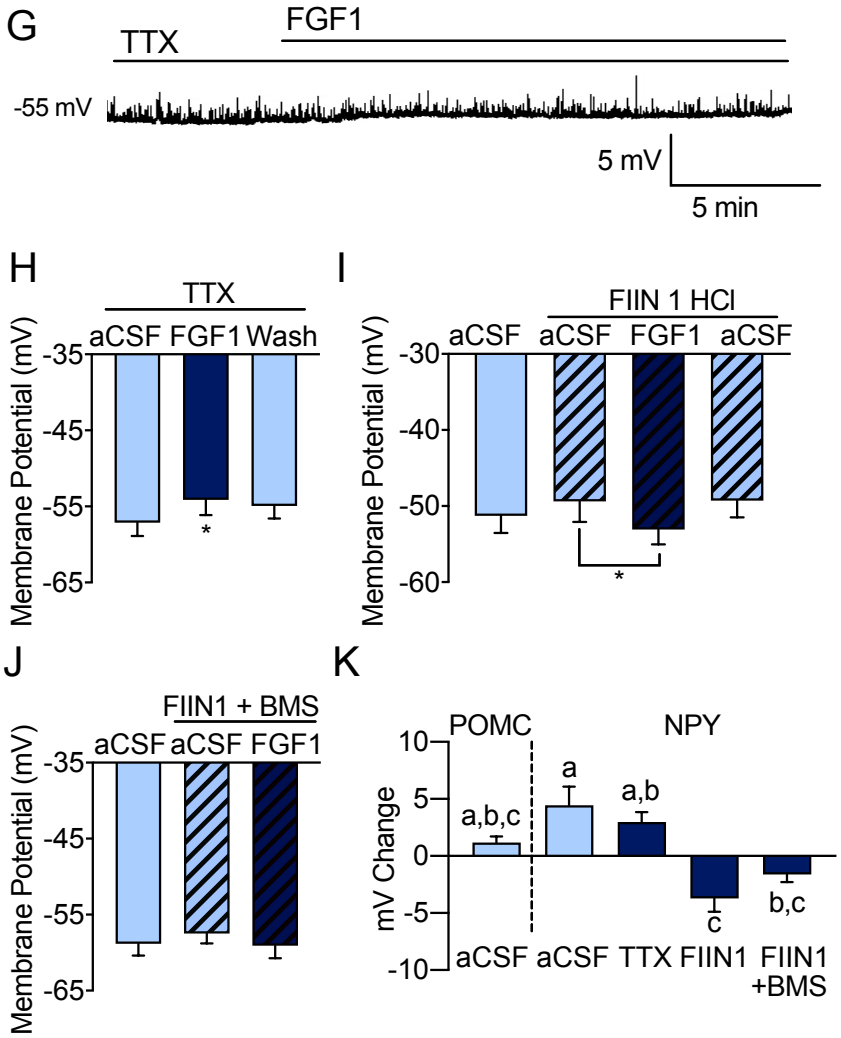


(*Unpublished data*)
Ritter, *Brain Res.* 2000
Tennant K, *Diabetes* 2019

FGF1 actions on NTS-NPY-GFP neurons

- FGF1 effects are direct
- Mediated by FGF and VEGF receptors





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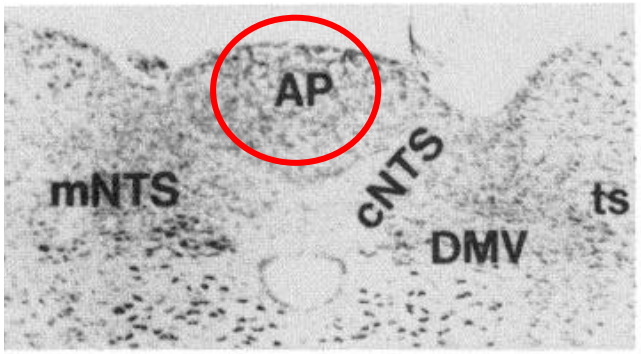
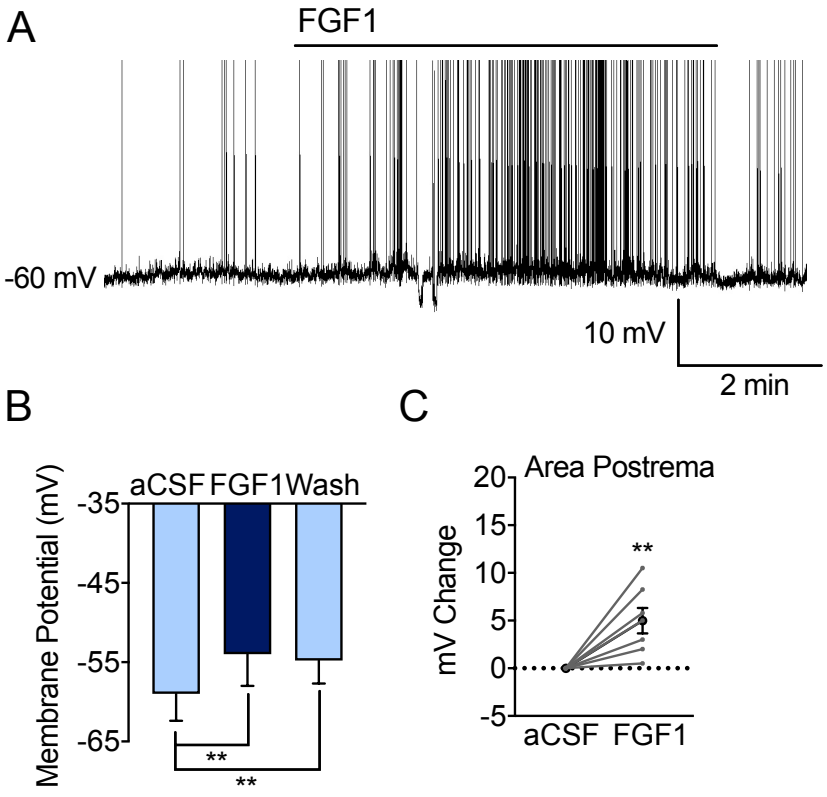
FGF1 activates unidentified neurons in the area postrema





(*Unpublished data*)

Summary

In the dorsal vagal complex (DVC):

- FGF1 has direct actions on NTS-NPY neurons
- FGF1 actions on NTS-NPY neurons are mediated FGF and VEGF receptors
- FGF1 activates unidentified neurons in the area postrema

Implications

- FGF1 electrophysiological actions in ARC consistent with proposed glia/astrocyte mediated mechanisms
- cFos expression and direct electrophysiological actions on NTS/AP neurons consistent with DVC control of glucoregulation and food intake

Do FGF1 central actions on food intake and blood glucose require peripheral vagal innervation from the DVC?

Acknowledgements

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- Rene Lindsley
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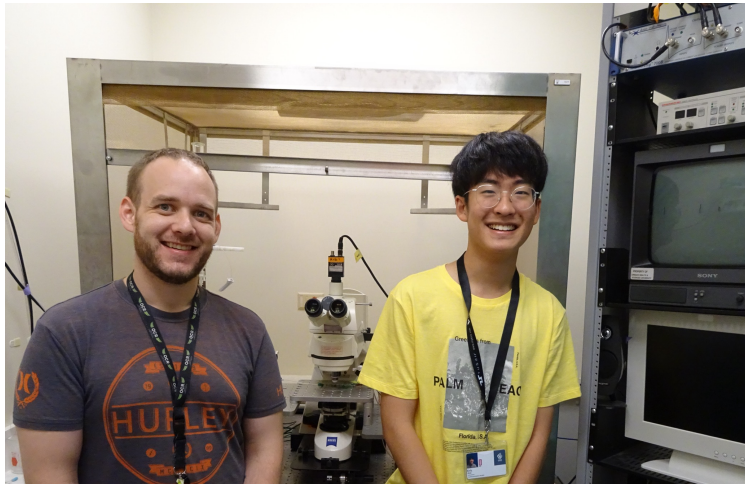
- Eric Kim

Martin Kelley

Animal Technician Staff

Ilia Karatsoreos



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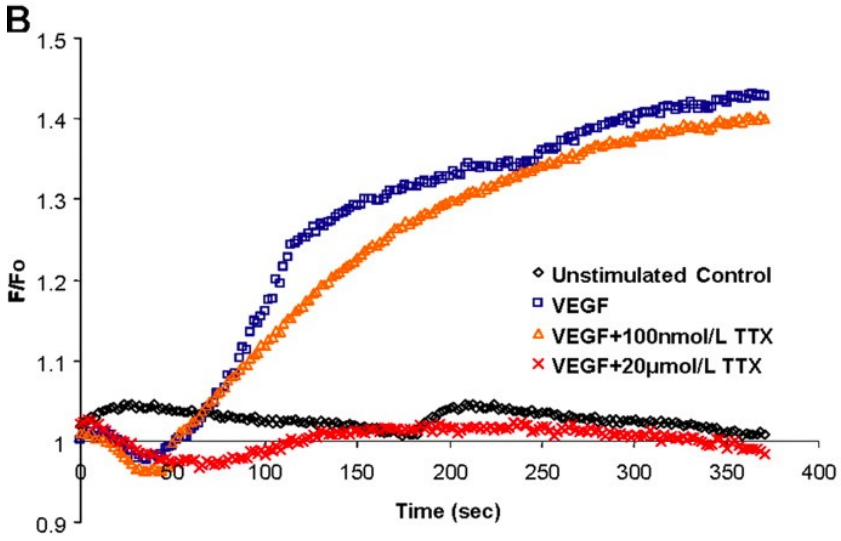
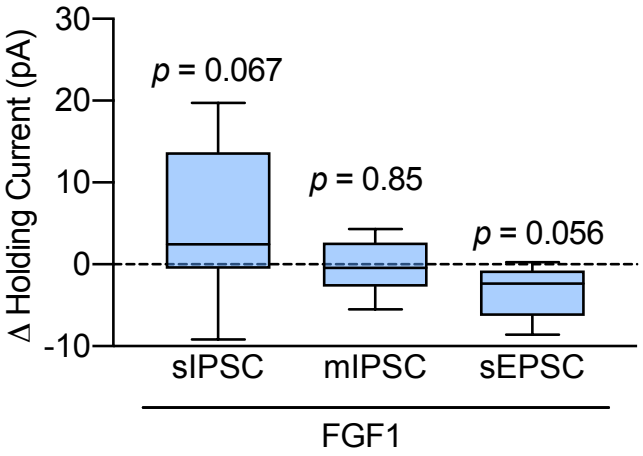
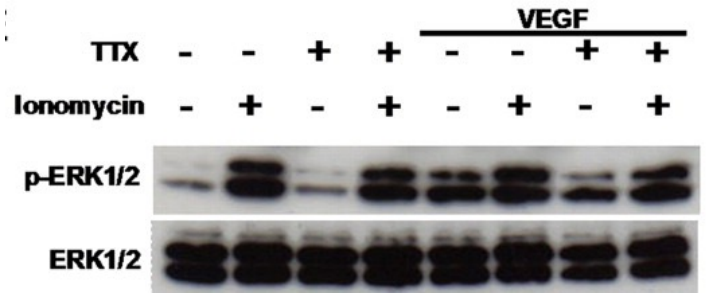
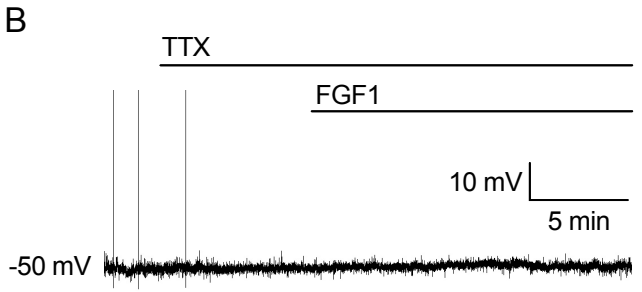
Questions?

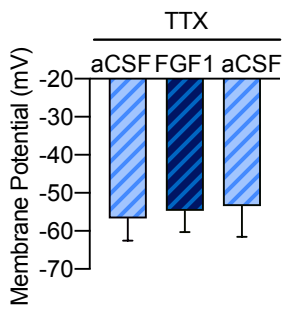
FGF1 effects may be mediated by voltage gated sodium channels (VGSC)



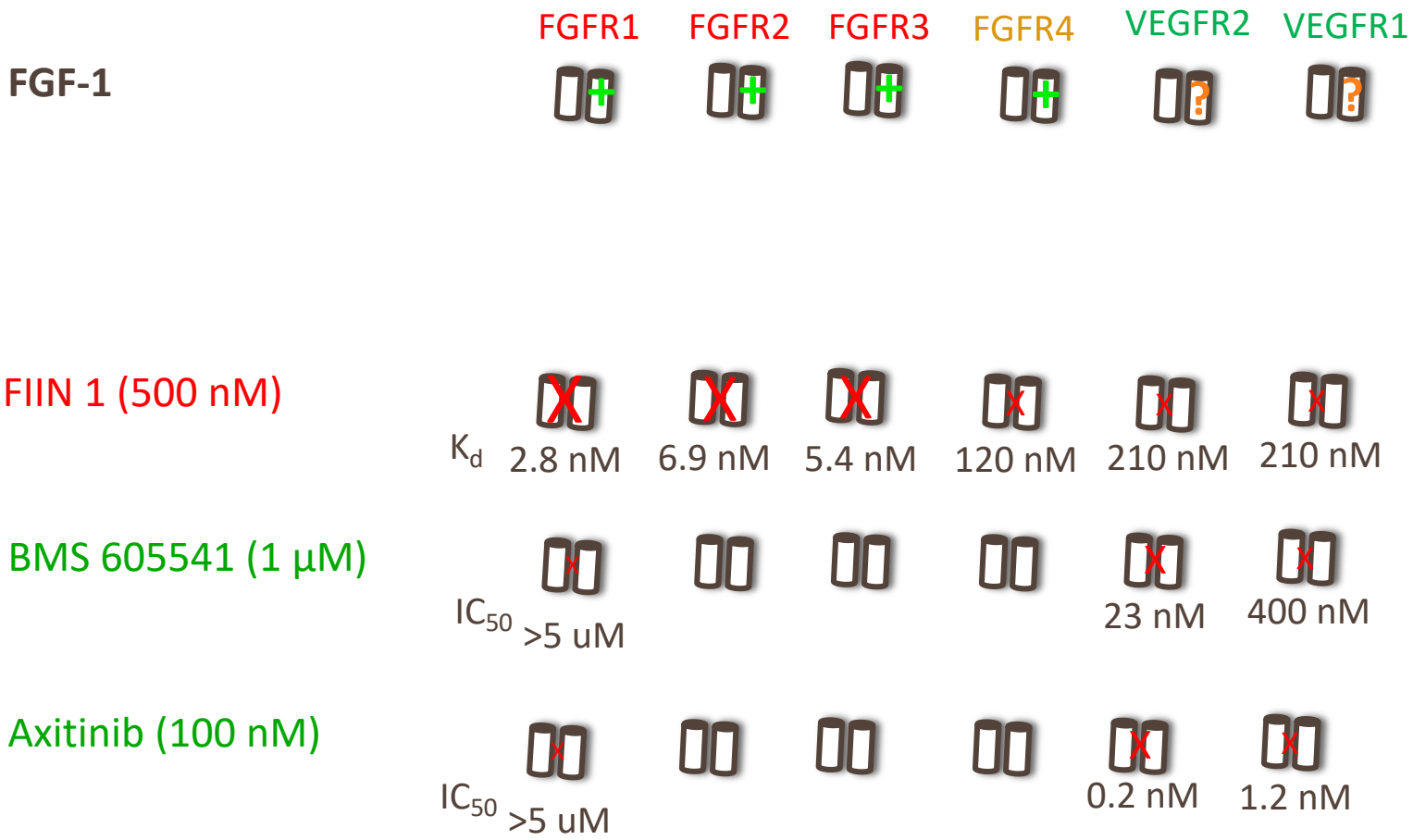








Andrikopoulos P. *J Bio Chem* 2011
(*unpublished data*)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VEGFR2
VEGFR1
FGF-1
+
+
+
+
?
?
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X
X
X
X
X
X
Kd
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X
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