School of Medicine
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The causes and consequences of recurrent hypoglycaemia in diabetes

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Disclosures

Advisory Panel: Sanofi, NovoNordisk

Board Member: NHS Tayside Health Board

Consultant:

Employee: University of Dundee

Other: NHS Tayside Health Board

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Stock/Shareholder None

Hypoglycaemia: Causes and Consequences

- Introduction
  - Hypoglycaemia and Impaired Awareness of Hypoglycaemia
- Hypoglycaemia detection
  - Where?
  - How?
  - Why?
- Habituation
  - The Good and the Bad
- Consequences of recurrent hypoglycaemia
  - The Ugly
    - Brain
    - Heart
- Conclusions

International Hypoglycaemia Study Group (IHSG) classification of hypoglycemia

Level 1
A glucose alert value of 3.9 mmol/L (70 mg/dL) or less. This need not be reported routinely in clinical studies, although this would depend on the purpose of the study
Level 2
A glucose level of <3.0 mmol/L (<54 mg/dL) is sufficiently low to indicate serious, clinically important hypoglycemia
Level 3
Severe hypoglycemia, as defined by the ADA, denotes severe cognitive impairment requiring external assistance for recovery

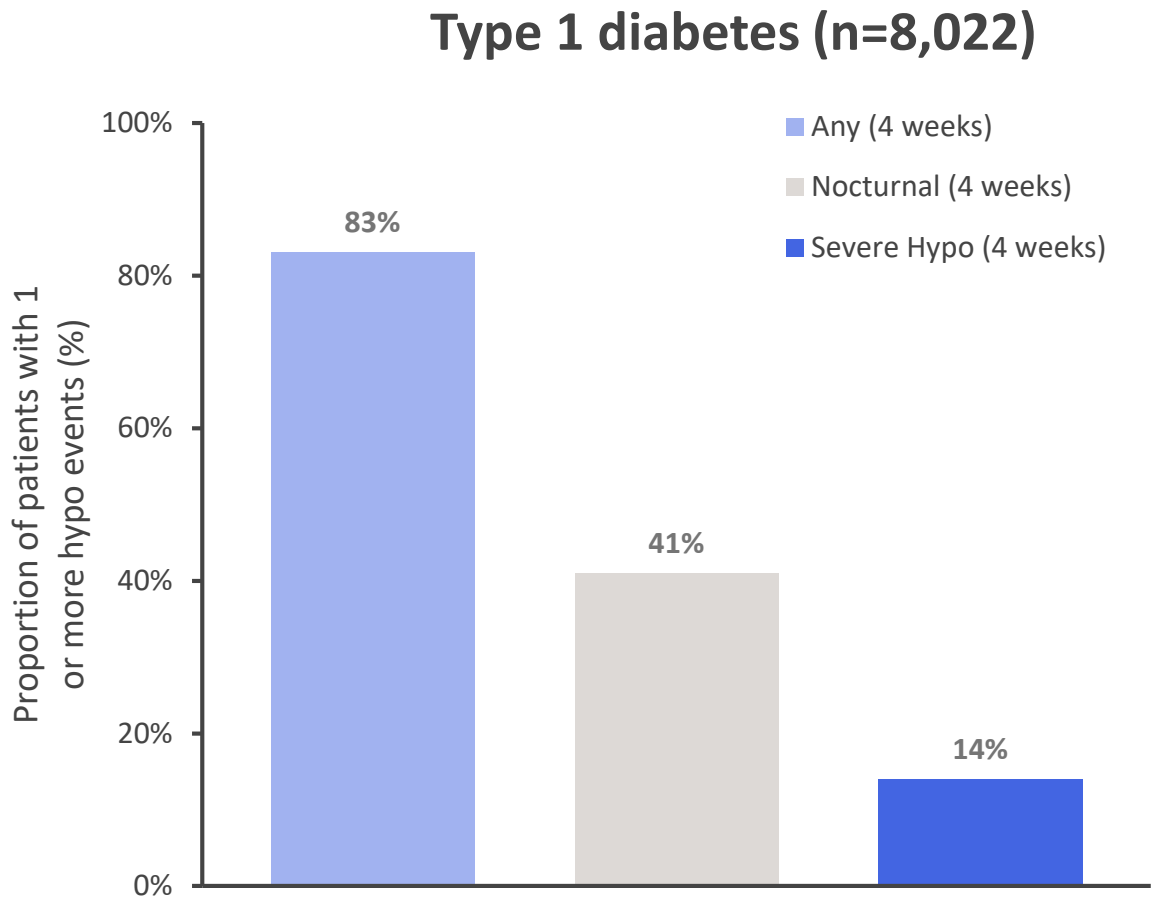
Diabetes Care 2017 Jan; 40(1): 155-157. https://doi.org/10.2337/dc16-2215

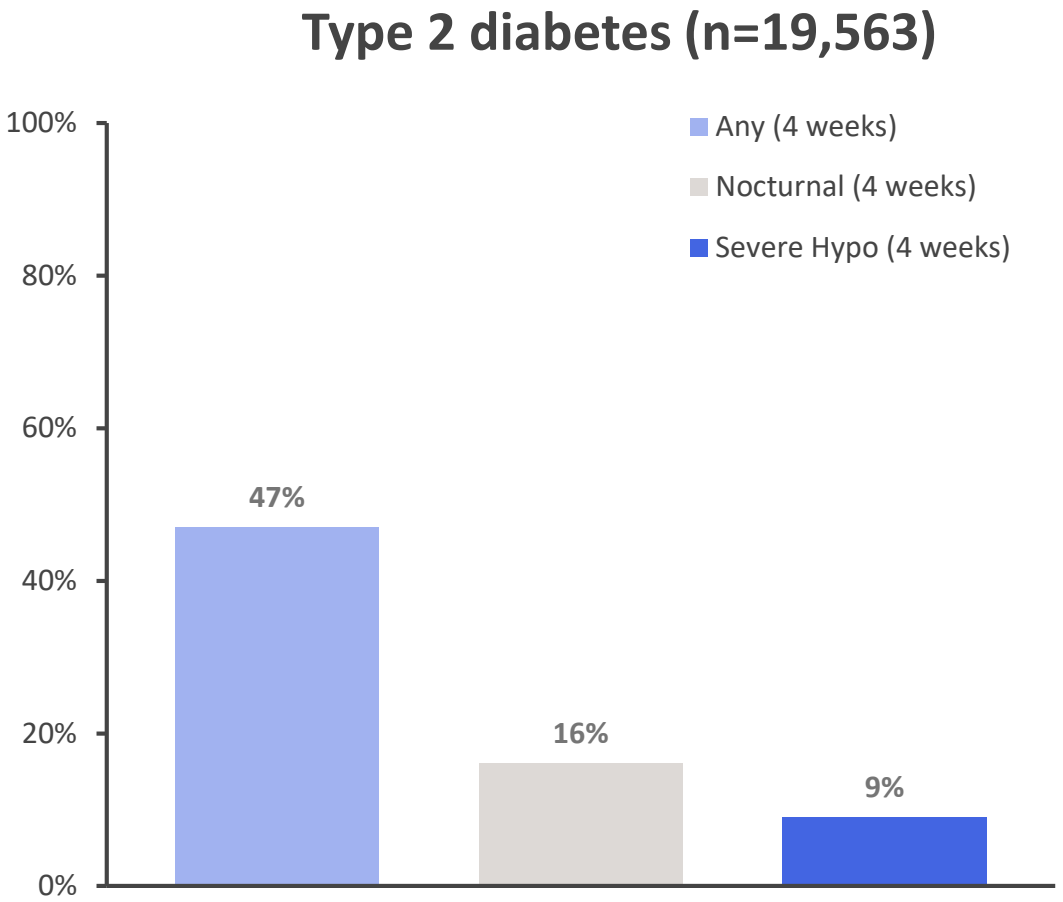
The International Hypoglycaemia Study Group Diabetologia (2017) 60: 3. https://doi.org/10.1007/s00125-016-4146-6

The size of the problem

Severe hypoglycaemia	Mild symptomatic hypoglycaemia	Asymptomatic hypoglycaemia	Nocturnal hypoglycaemia
• T1DM: at least one episode per patient-year; 20% with recurrent episodes	• T1DM: up to 2 episodes per patient-week	• Increasingly evident from CGM; up to 75% of all events in T1DM	• Although reduced by the use of long-acting insulin analogues - still frequent
• Insulin-treated T2DM: one third of that in T1DM	• Insulin-treated T2DM: one third of that in T1DM		

HAT study results: Hypoglycaemia is significantly underreported

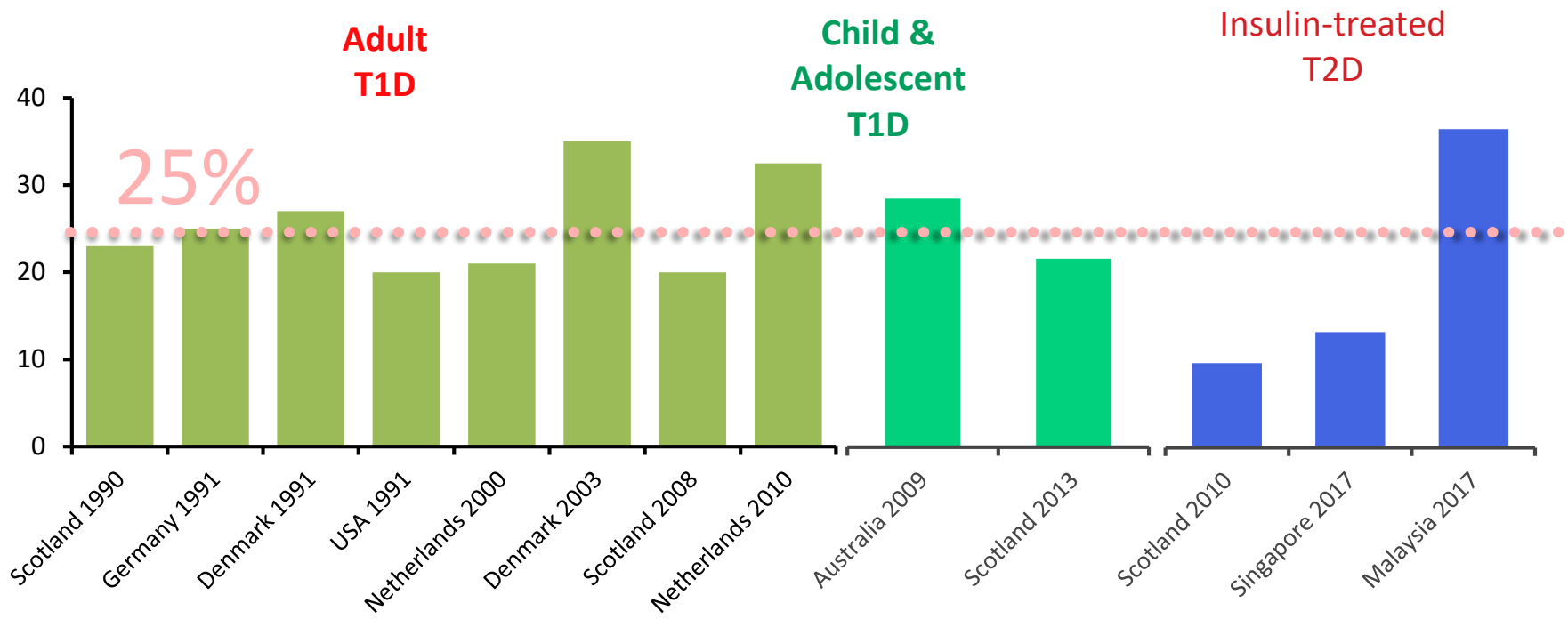




Rates and prevalence calculated from full analysis set.

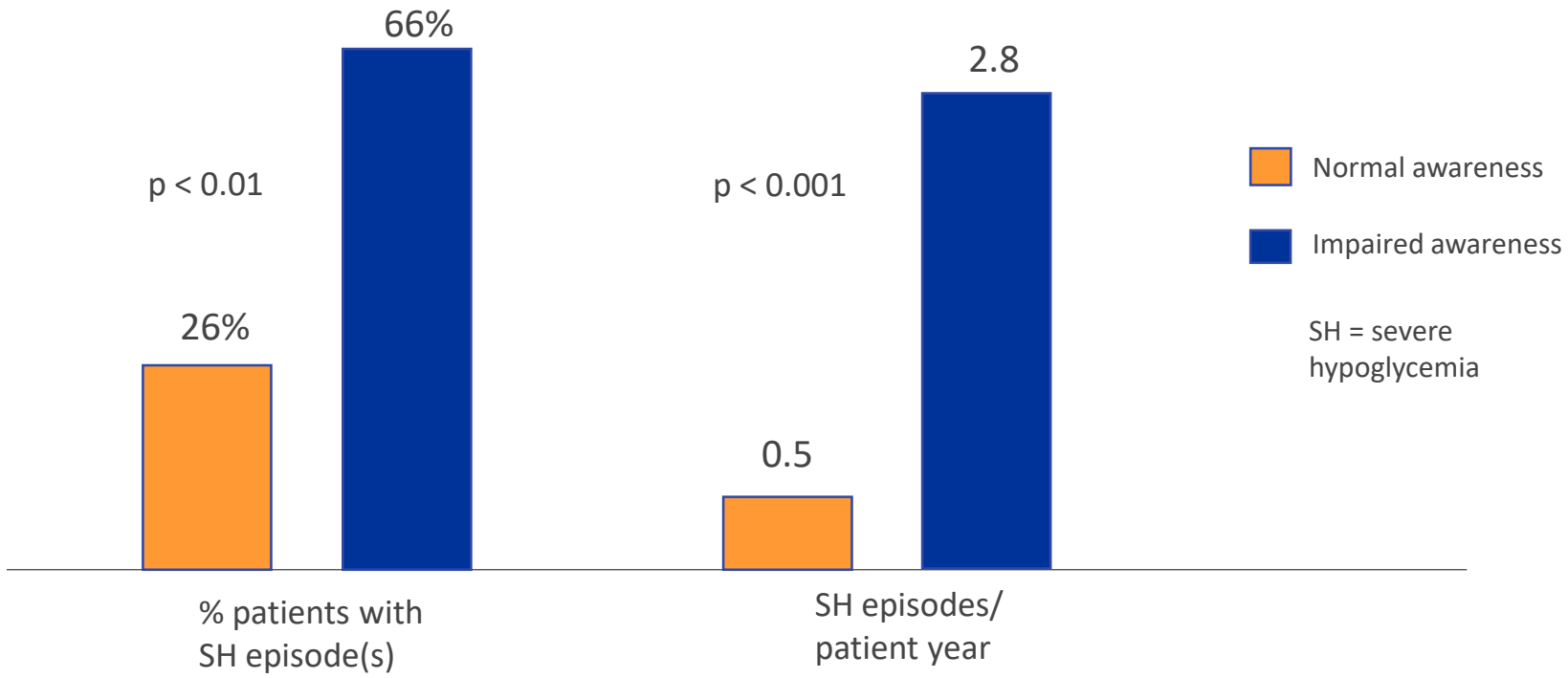
Khunti K, Alsifri S, Aronson R, et al. Self-reported hypoglycaemia: a global study of 24 countries with 27,585 insulin-treated patients with diabetes: the HAT study. *Diabetologia* 2014; 57(Suppl.1):S201.

Prevalence of Impaired Awareness of Hypoglycaemia in Insulin-treated Diabetes



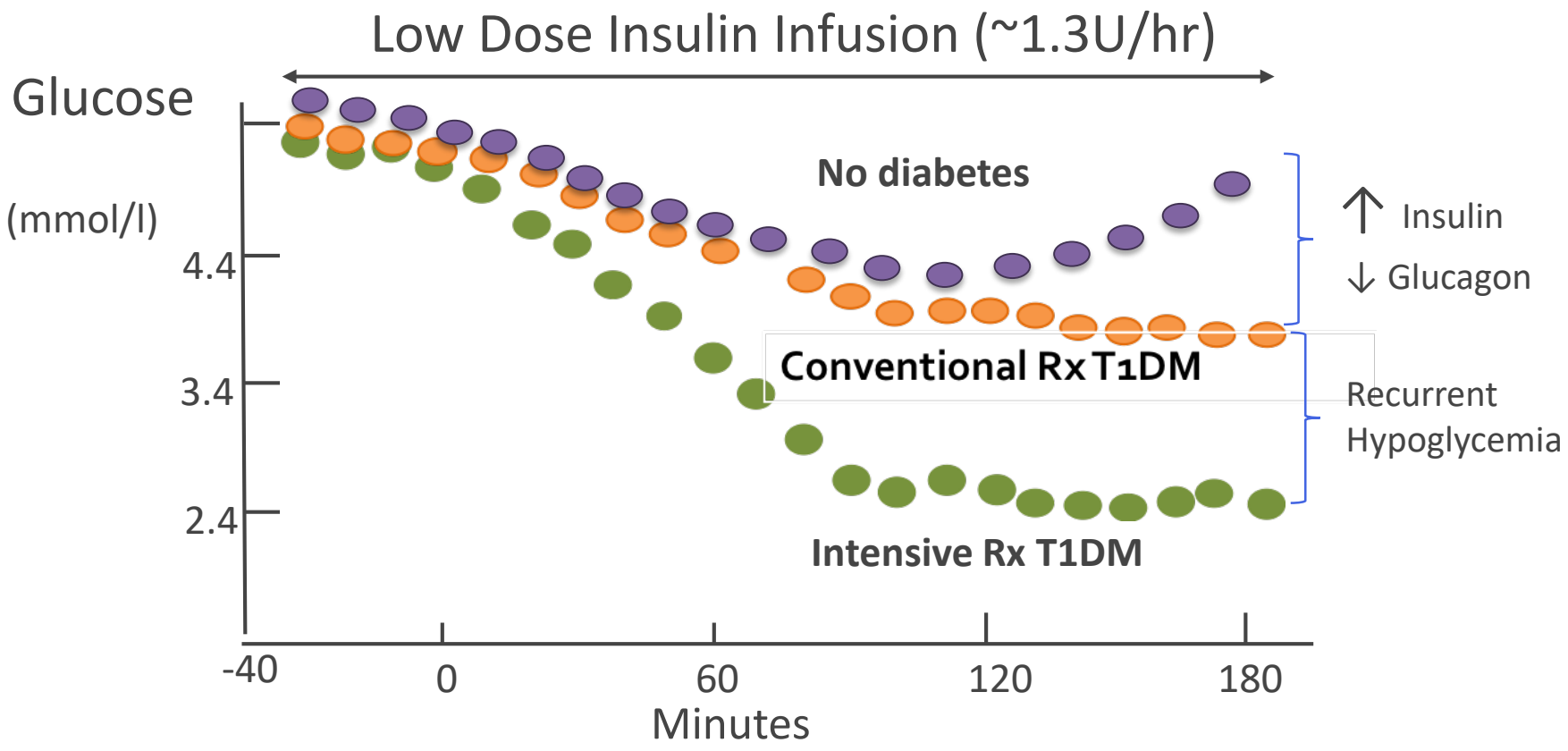
Hepburn ea, *Diabet Med* 1990; Muhlauser ea *Diabetes Care* 1991, Pramming ea, *Diabet Med* 1991, Orchard ea, *Diabet Med* 1991, ter Braak, *Diabetes Care* 2000, Pedersen-Bjergaard ea, *Diabetologia* 2003, Geddes ea, *Diabet Med* 2008, A' Campo ea, *Diabetologia* 2010.

Impact of IAH on severe hypoglycemia



Gold AE et al. Frequency of Severe Hypoglycemia in Patients With Type I Diabetes With Impaired Awareness of Hypoglycemia. Diabetes Care 1994;7:697-703.

Glucose Counterregulation in people with and without type1 diabetes



Amiel et al Diabetes. 1988 Jul;37(7):901-7

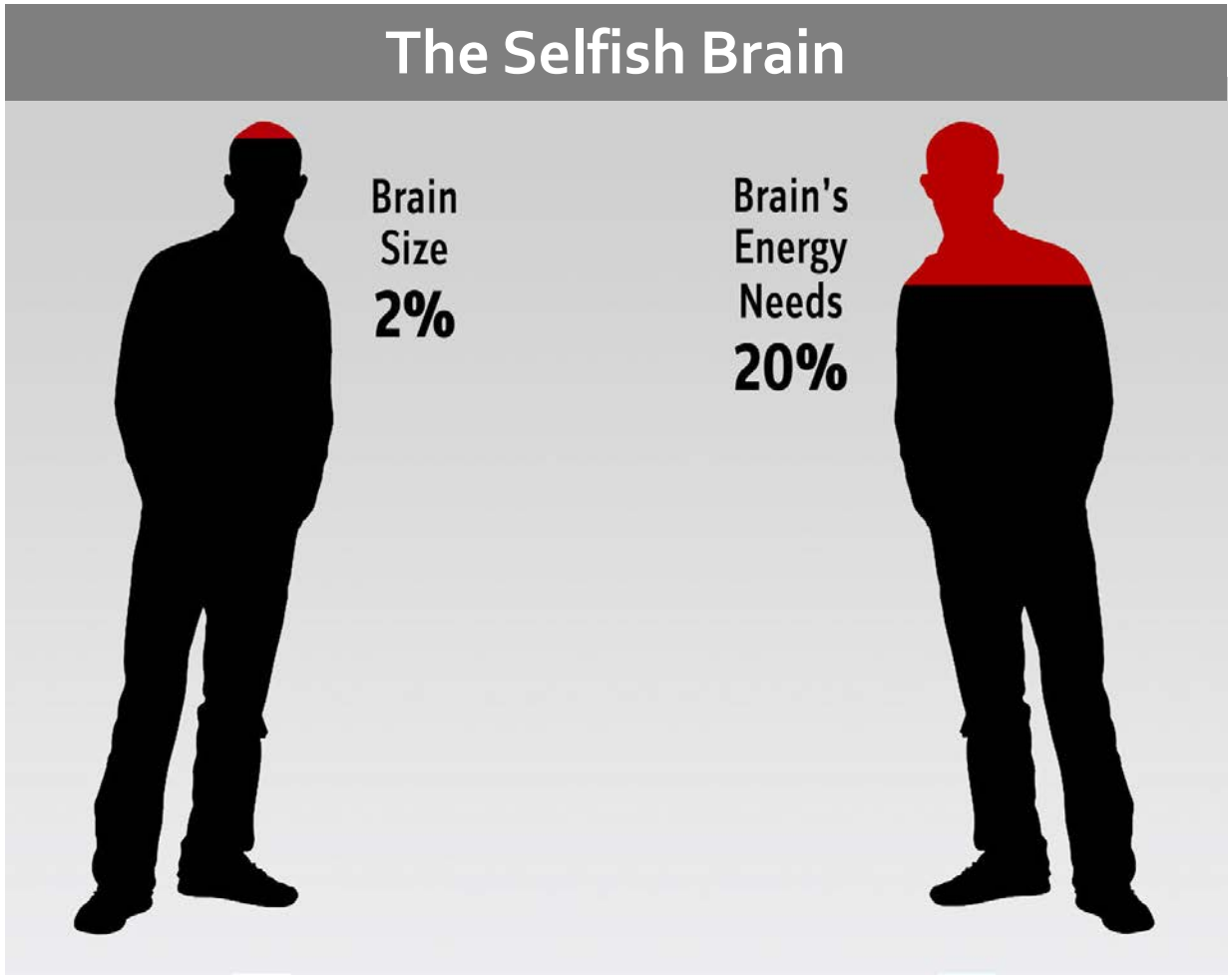
Central Mechanisms of Hypoglycemia Awareness

1. Where do we sense low glucose ?

2. How do we sense low glucose ?

3. What are the central mechanism that lead to impaired awareness of hypoglycemia?

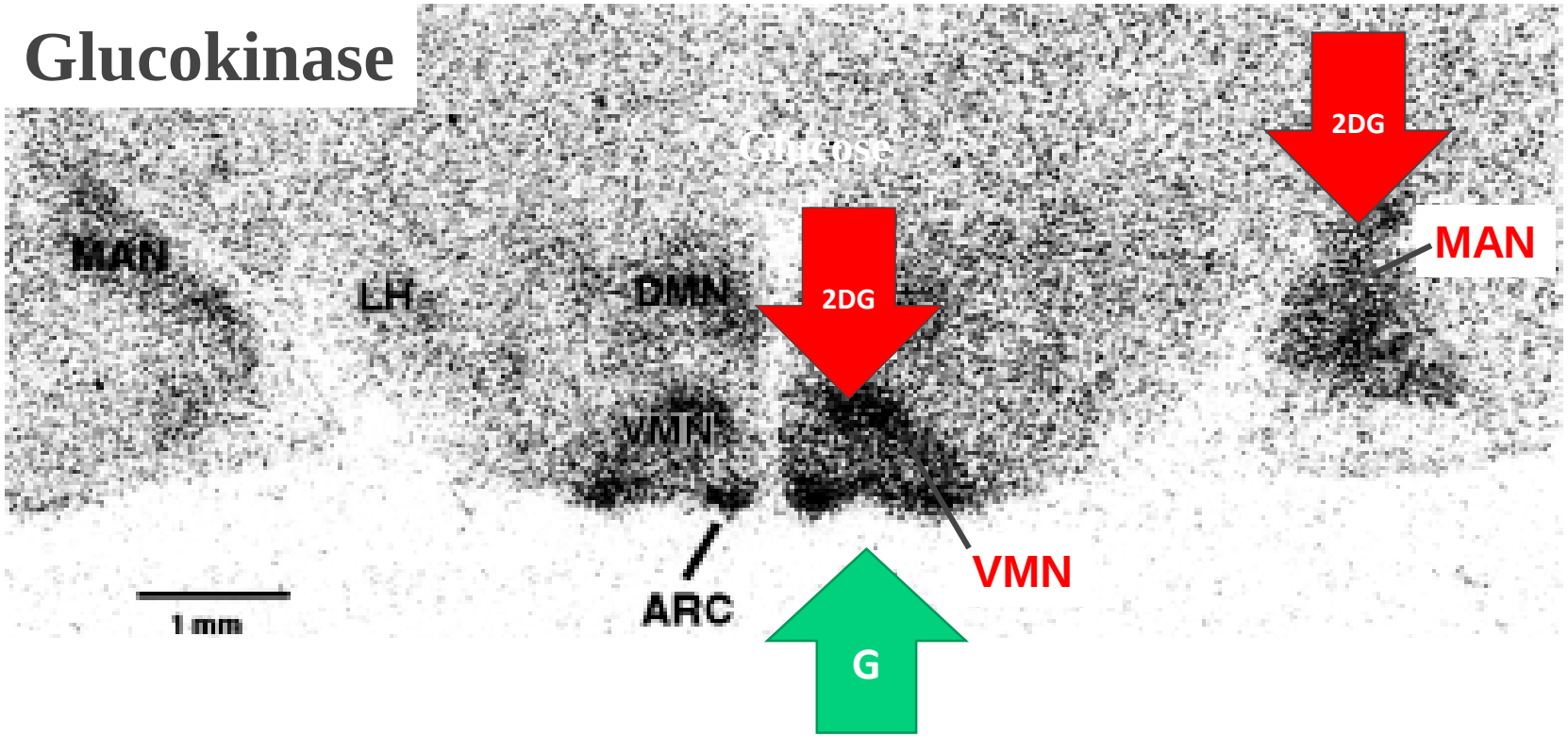
Where do we sense glucose?



Glucose sensing in multiple brain regions

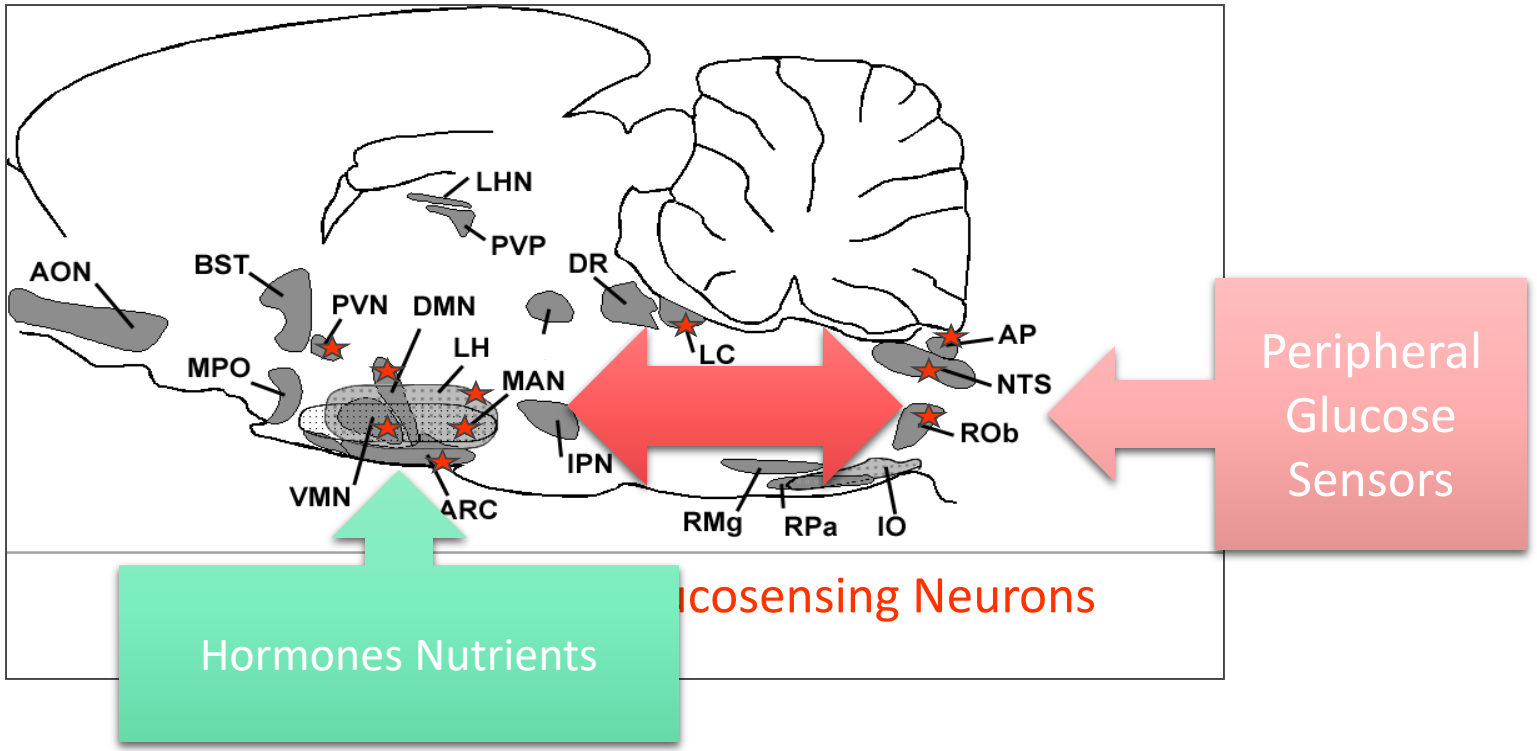
Borg et al. Diabetes 99(2):361-65, 1997

Zhou et al. Diabetes 59(10):2646-52, 2010



Adapted from Lynch, Tompkins, Brooks, Dunn-Meynell and Levin, Diabetes 2002

Neural Circuitry for Detection of Hypoglycemia



Stanley et al. Endocr Rev, Volume 40, Issue 3, June 2019, Pages 768–788, https://doi.org/10.1210/er.2018-00226

Dunn-Meynell *et al. Diabetes* 2002;51:2056
Maekawa *et al. Endocrinol 200;*141:375

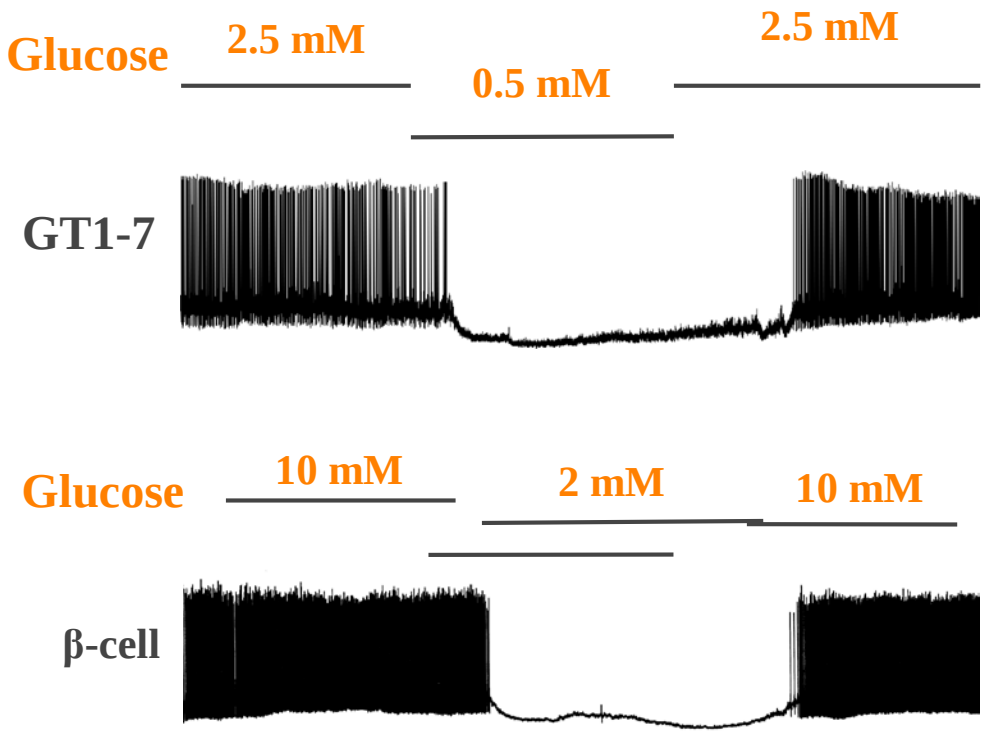
Central Mechanisms of Hypoglycemia Awareness

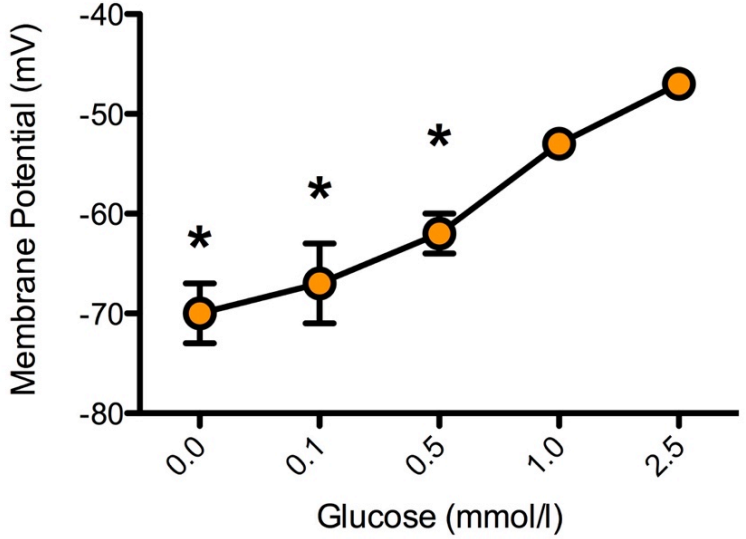
1. Where do we sense low glucose ?
    1. Interconnected circuit of specialized neurons found in multiple brain regions and in the periphery

2. How do we sense low glucose ?

3. What are the central mechanism that lead to impaired awareness of hypoglycemia?

Hypothalamic Glucose-sensing Neurons



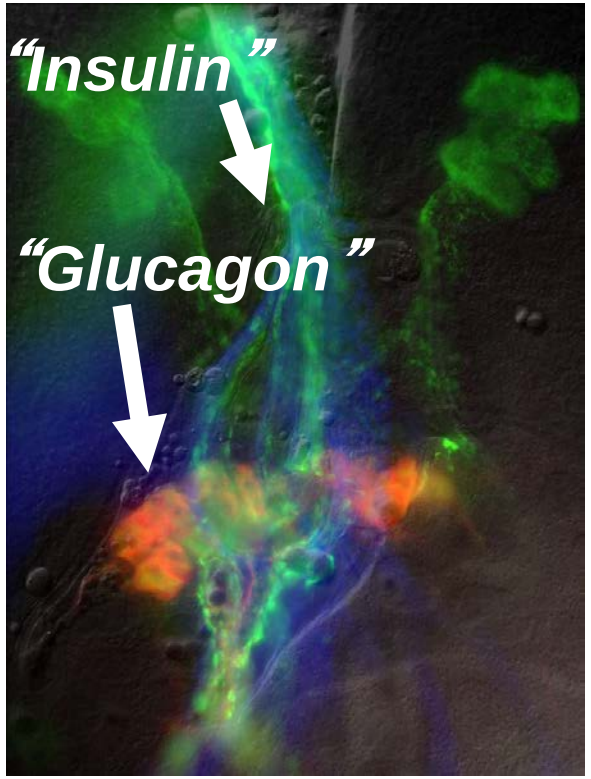


Beall et al. Diabetologia 2012

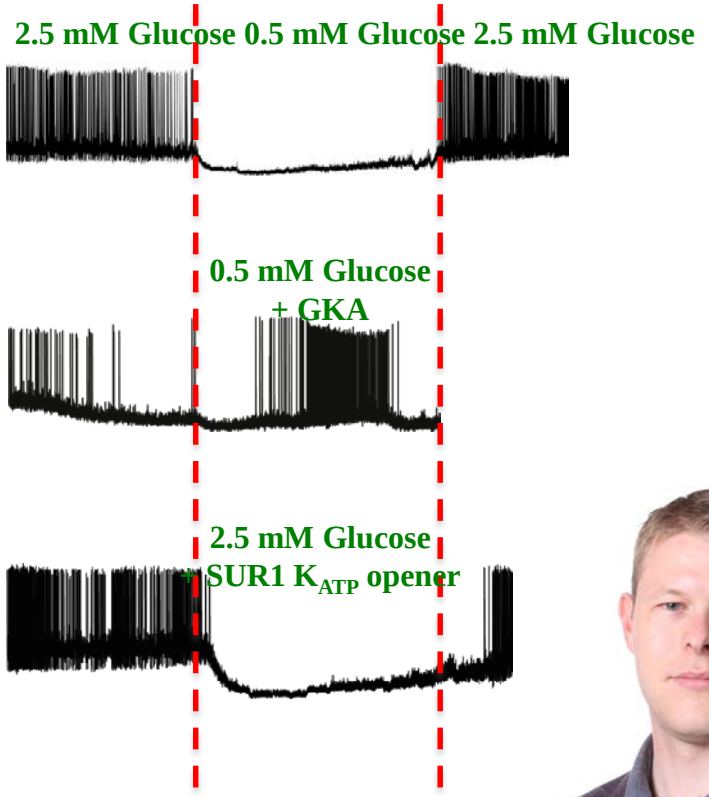
The Hypothalamic "Islet"

Glucose excited and glucose inhibited neurons paralleling ß- and α-cells

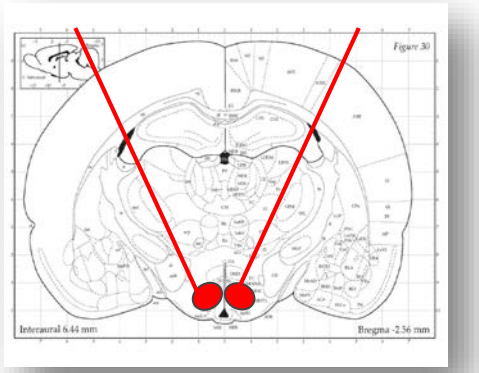
Fly Brain



Glucose sensing neuron

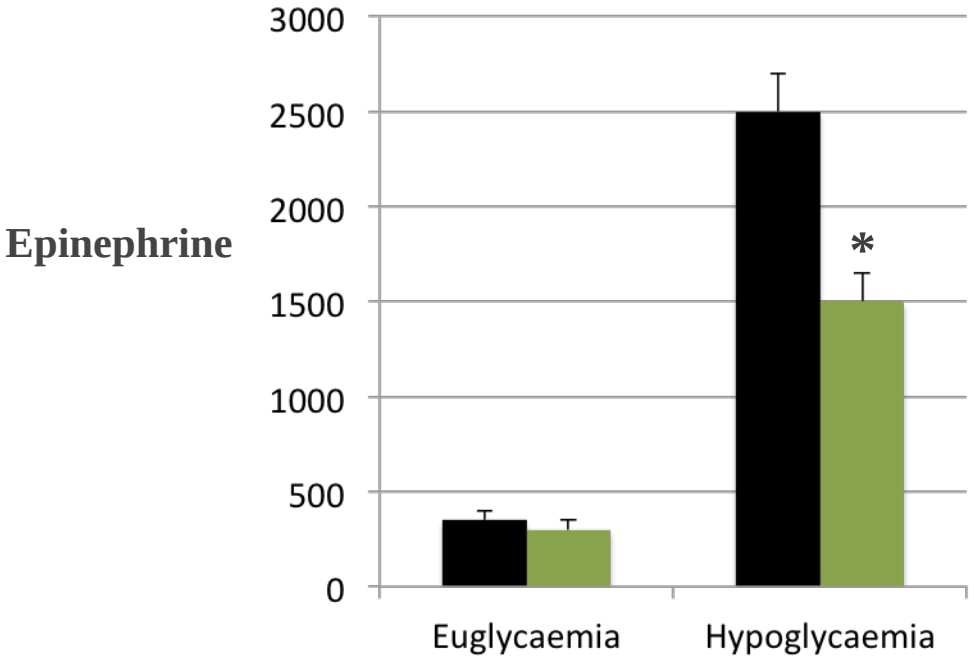


Beall et al. Diabetologia 2012



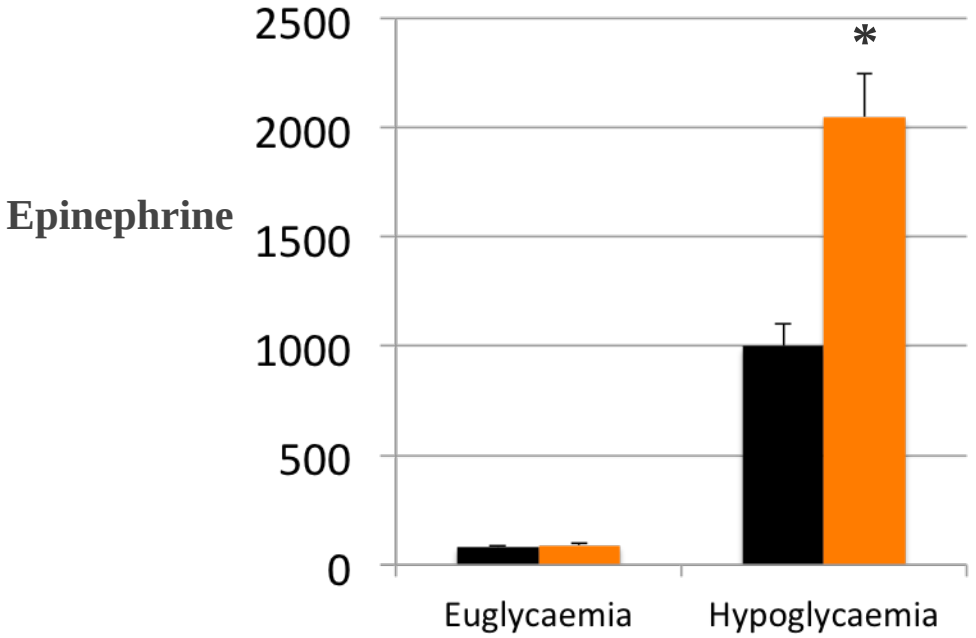
The K_{ATP} channel in Neuronal Glucose Sensing in vivo

K_{ATP} blocker: Glibenclamide



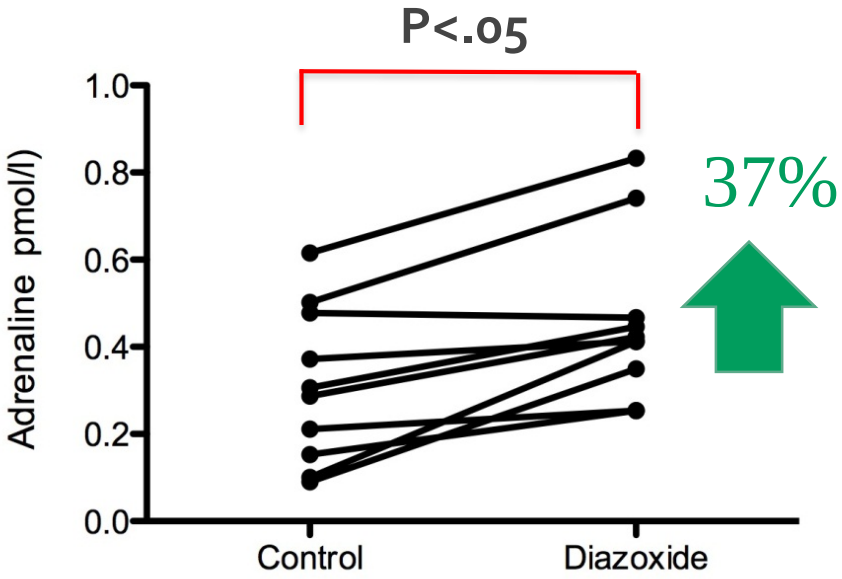
Evans & McCrimmon et al. Diabetes 2004

K_{ATP} opener: NN414 /Diazoxide



McCrimmon et al. Diabetes 2005

Oral Diazoxide Amplifies the Counterregulatory Response to Hypoglycemia in Type 1 diabetes



George et al Diabetes 2015 Jun; 64(6): 2234-2241.

Central Mechanisms of Hypoglycemia Awareness

1. Where do we sense low glucose ?
    1. Interconnected circuit of specialized neurons found in multiple brain regions and in the periphery

2. How do we sense low glucose ?
    - Glucose-Excited Neurons: **GK, AMPK, SUR-1 $K_{ATP,}$ GABA, SGLT, T1R2/3**
    - Glucose-Inhibited Neurons: **GK, SUR-1 $K_{ATP,}$ AMPK, Glutamate**

    2. What are the central mechanism that lead to impaired awareness of hypoglycemia?

Central Mechanisms of Hypoglycemia Awareness

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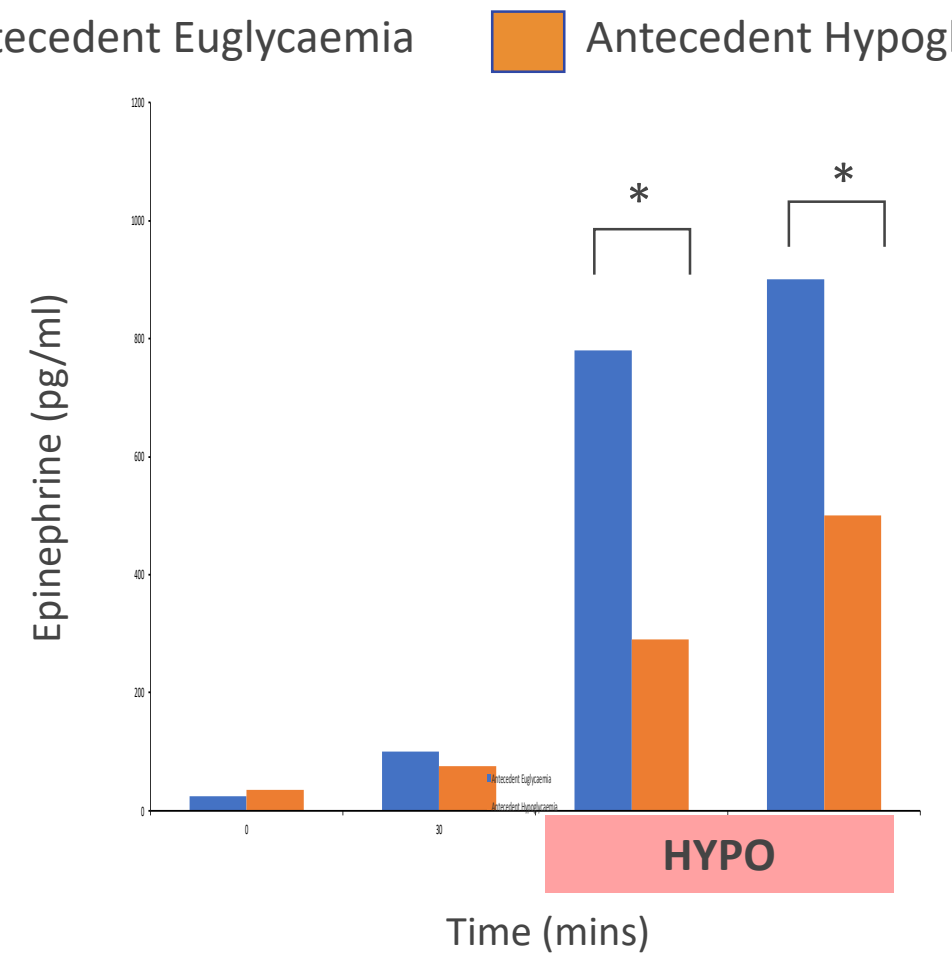
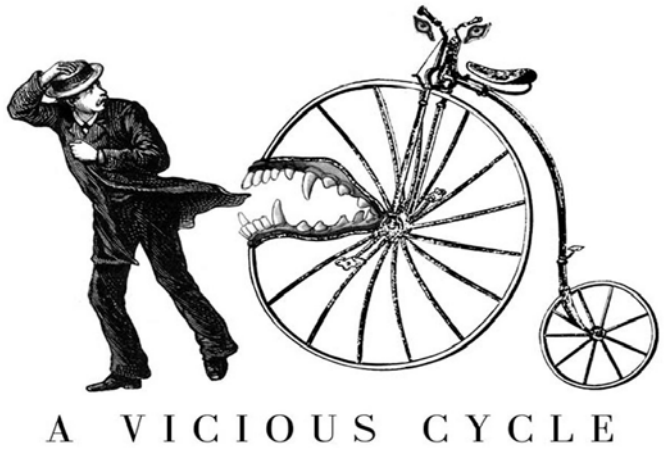
2. What are the central mechanism(s) that lead to impaired awareness of hypoglycemia?

Hypoglycaemia Impairs Neuroendocrine Responses To Subsequent Hypoglycaemia

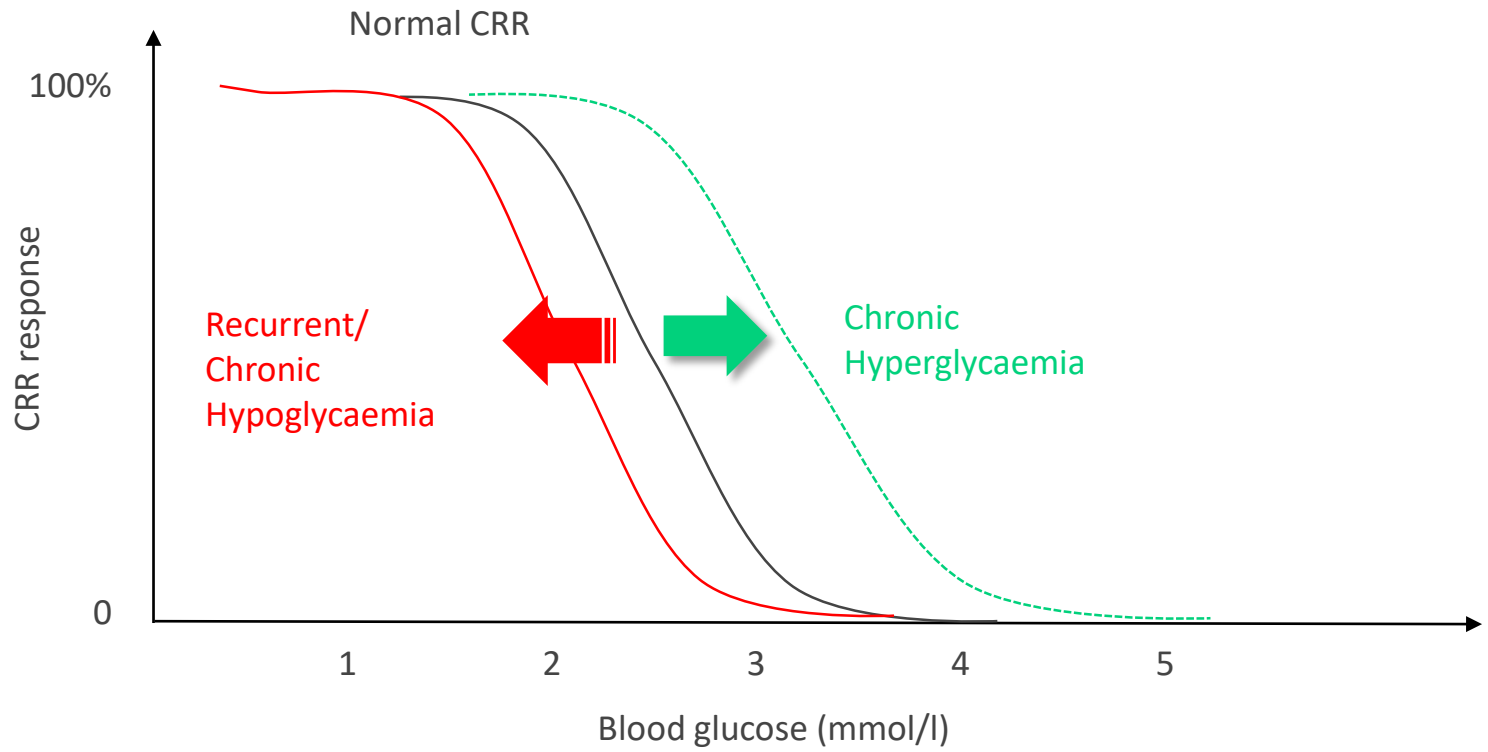


A maladaptive response to repeated hypoglycaemia Hypoglycemia-Associated Autonomic Failure

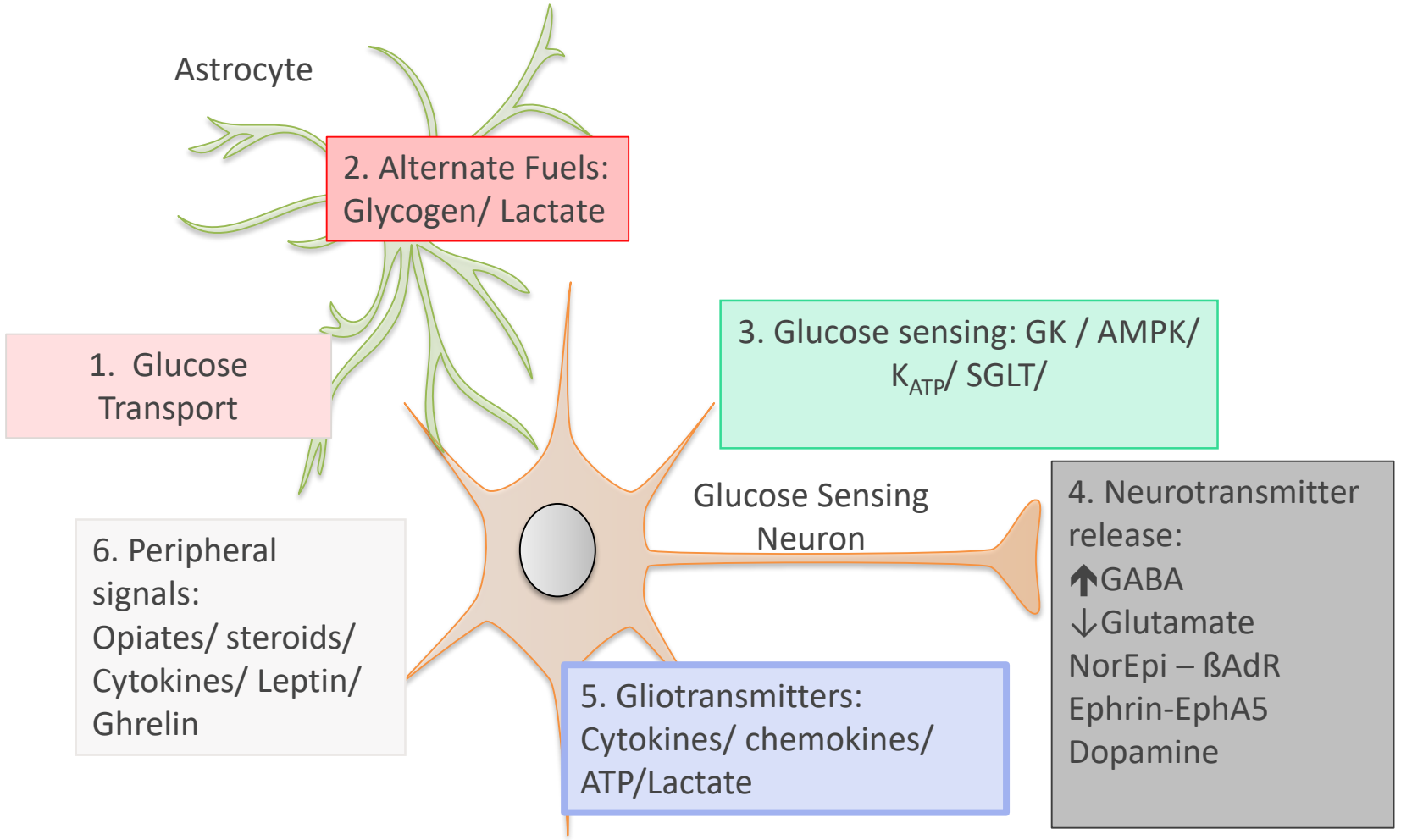


Adapted from Heller et al. *Diabetes* 1991

Shifting Thresholds For Hypoglycaemia Detection



Potential Mechanisms of cerebral adaptation to recurrent hypoglycemia



Good habits, bad habits and Aplysia

Habituation

"The reduction of psychological, behavioral or physiological response to a stimulus as a result of repeated or prolonged exposure"

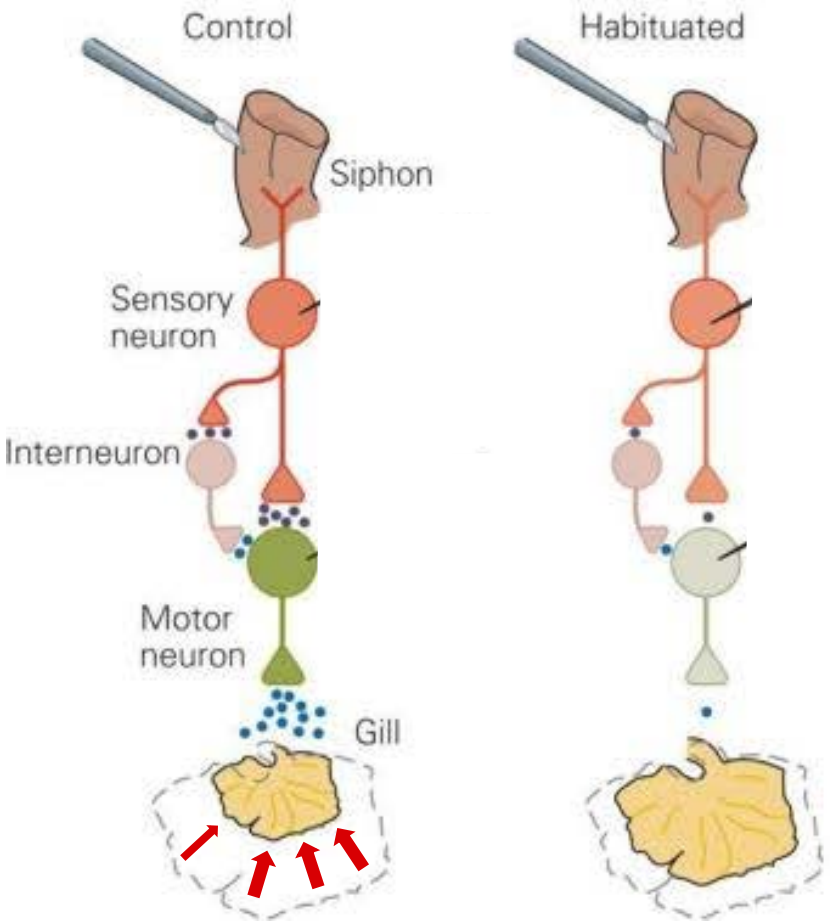
Thompson and Spencer 1966

Habituation

"The reduction of psychological, behavioral or physiological response to hypoglycaemia as a result of repeated or prolonged hypoglycaemia"

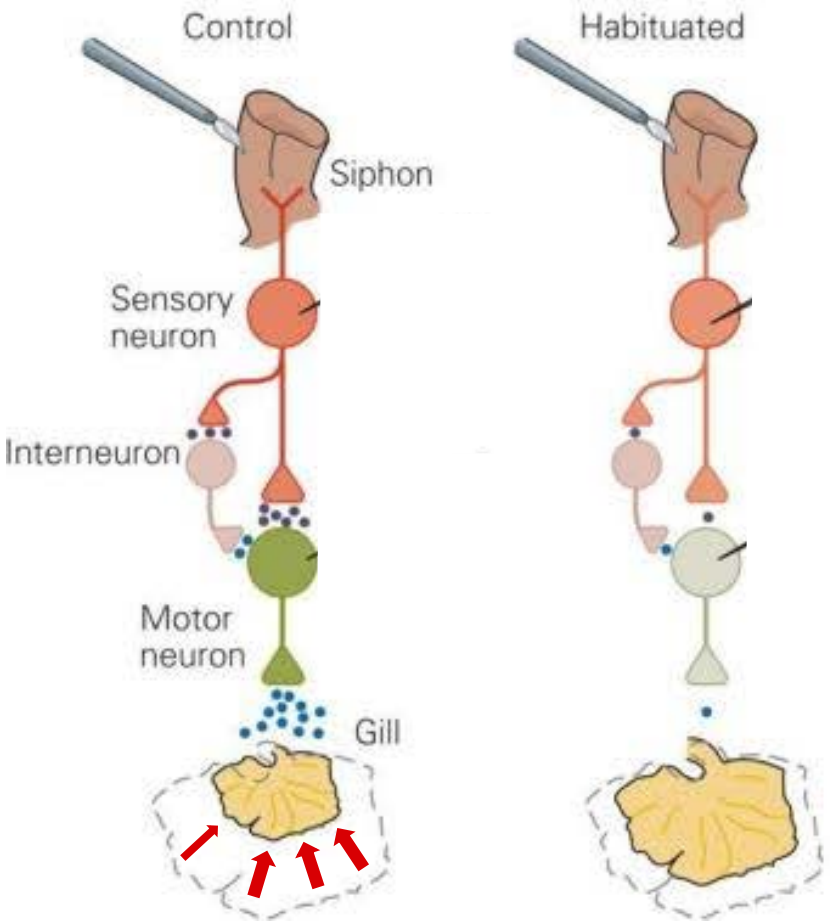
Thompson and Spencer 1966

Habituation: Gill Withdrawal Reflex in Aplysia

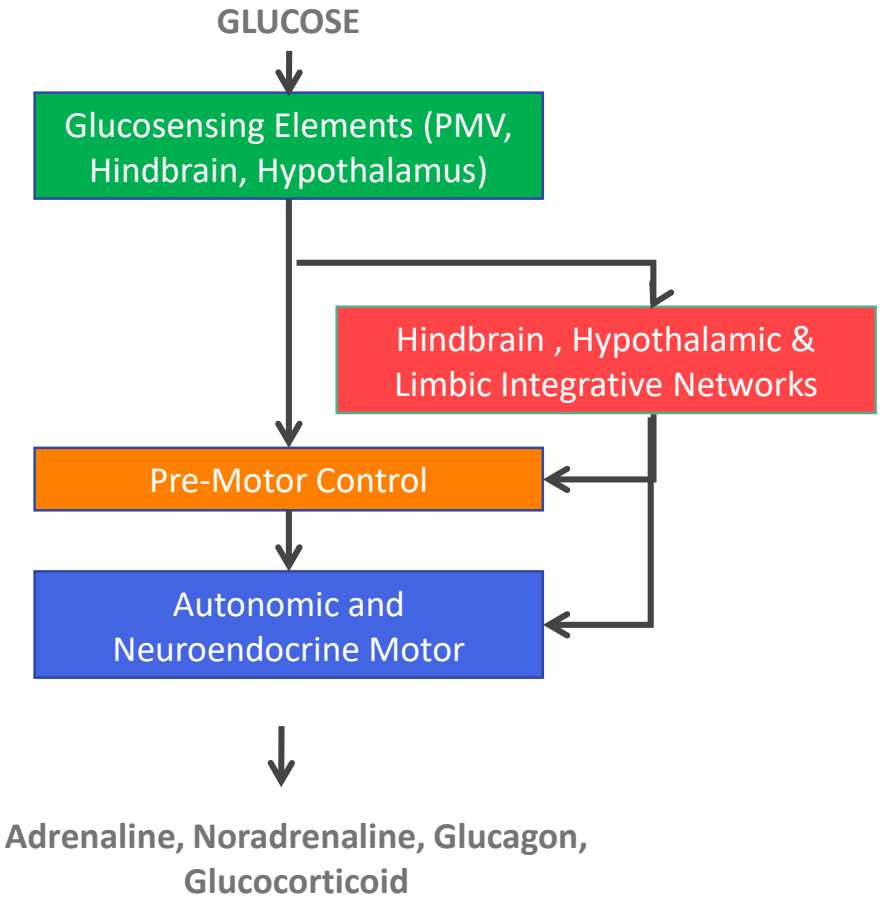


Gill withdrawal reflex

Habituation: Gill Withdrawal Reflex in Aplysia



Gill withdrawal reflex

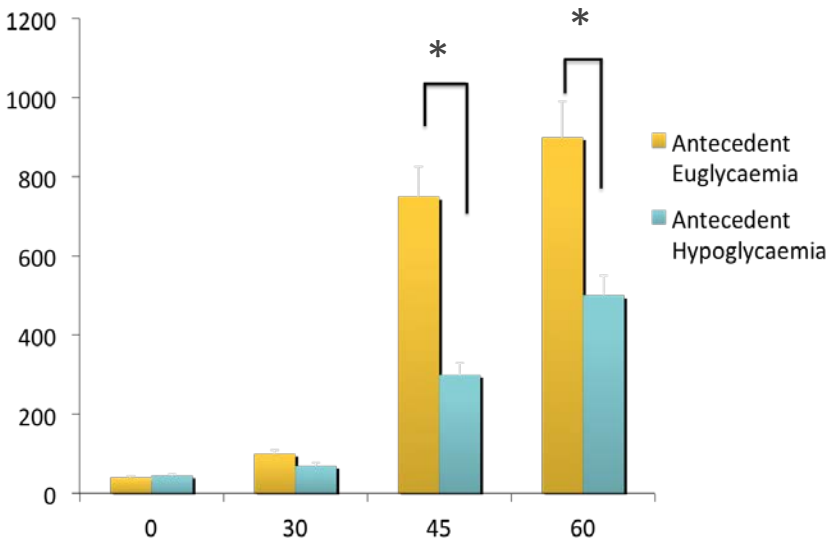


Watts & Donovan Front Neuroendocrinol. 2010 Jan;31(1):32-43

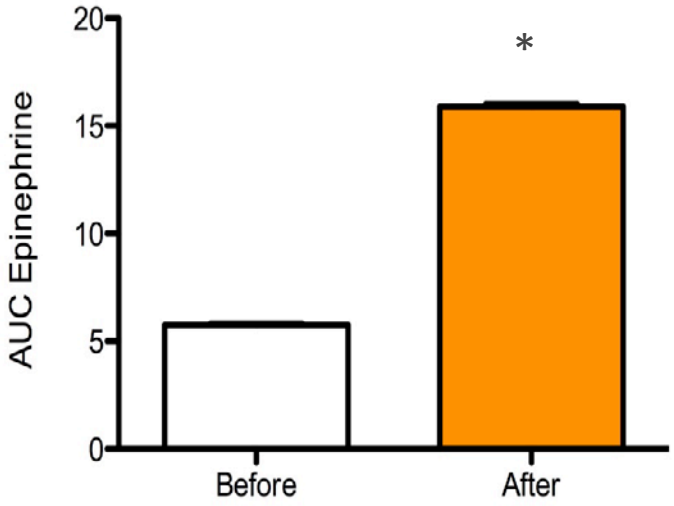
Habituation

Criteria 1: Given that a particular stimulus elicits a response, repeated applications of that stimulus result in a decreased response (Habituation)

Criteria 2: If the stimulus is withheld, the response tends to recover over time (spontaneous recovery)



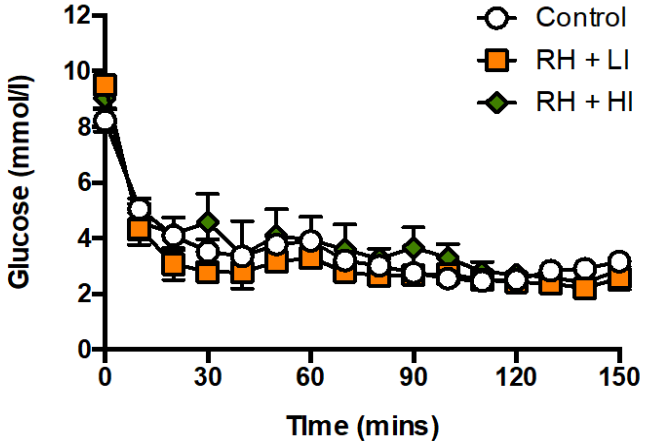
Adapted from Heller and Cryer Diabetes 1991

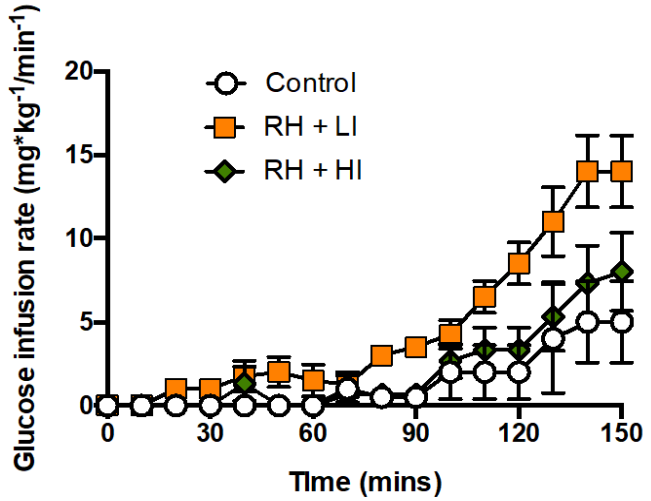


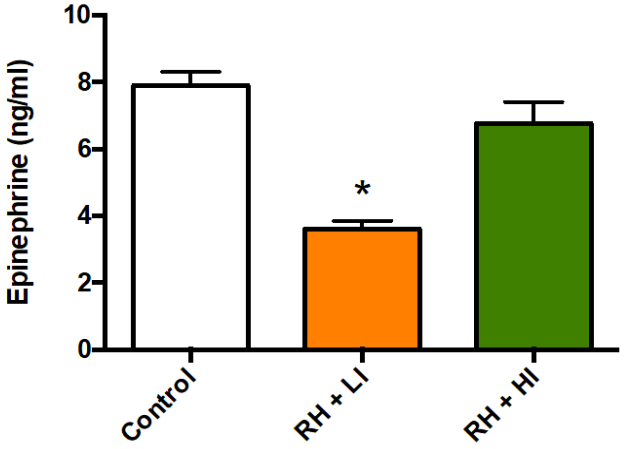
Cranston et al. Lancet 1994

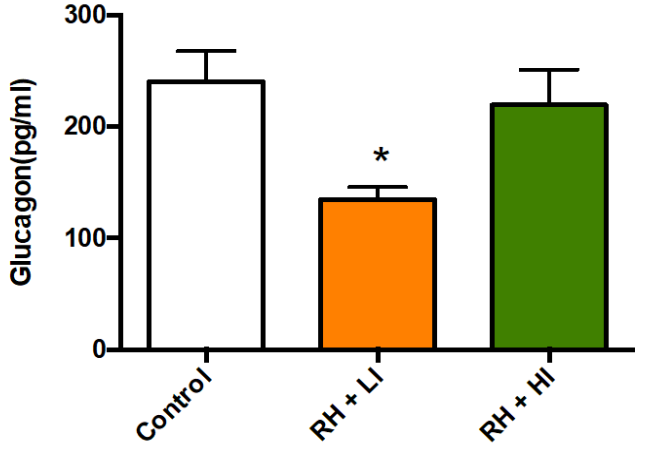
Hypoglycaemia Dishabituation with High Intensity Exercise: Rodents

Criteria 8: Presentation of another (usually strong) stimulus results in recovery of the habituated response (dishabituation)



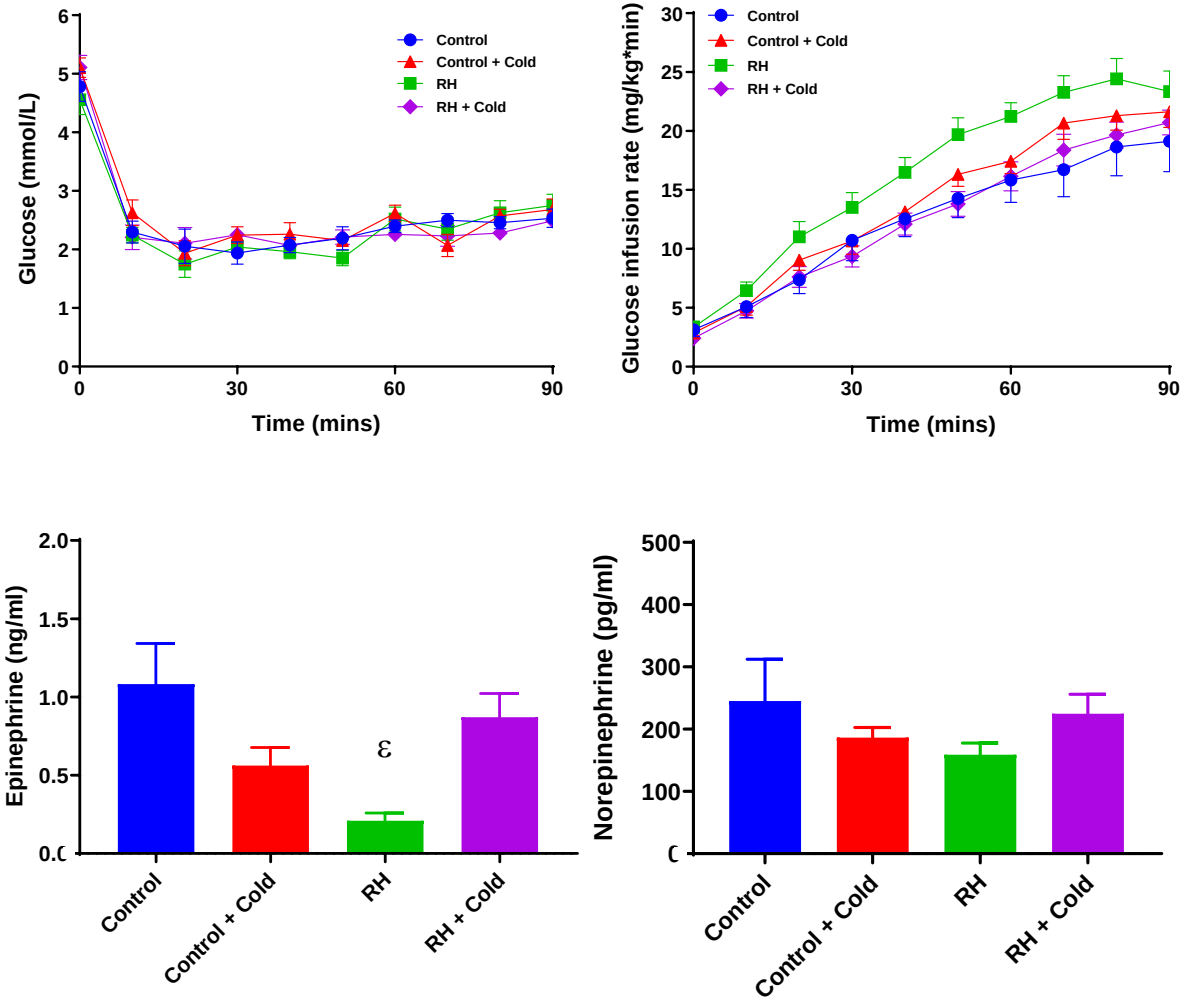






McNeilly, Diabetes 2017 66 (6), 1696-1702

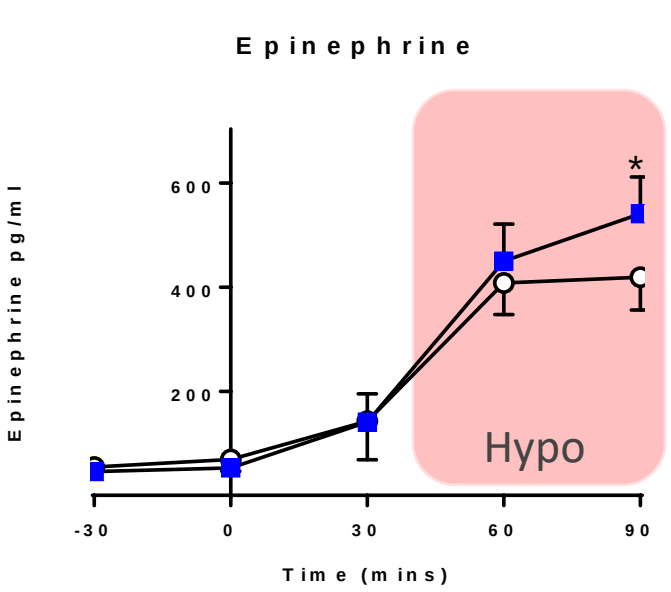
Hypoglycaemia Dishabituation with Cold Exposure

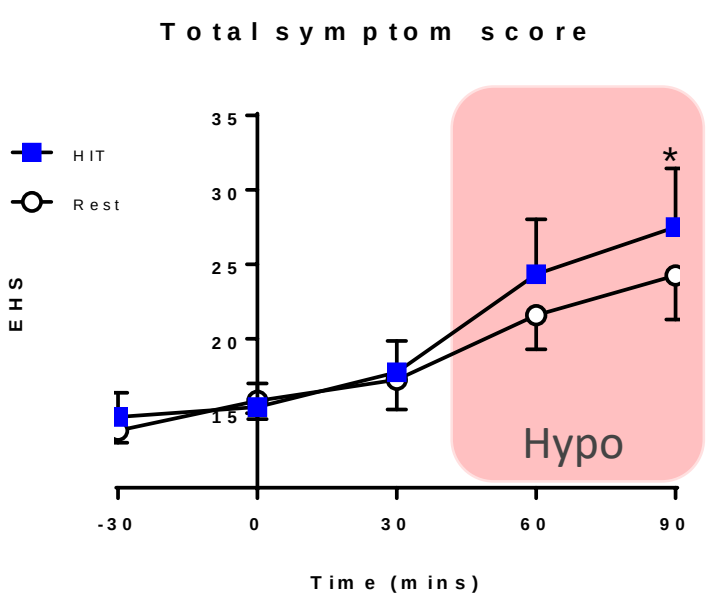


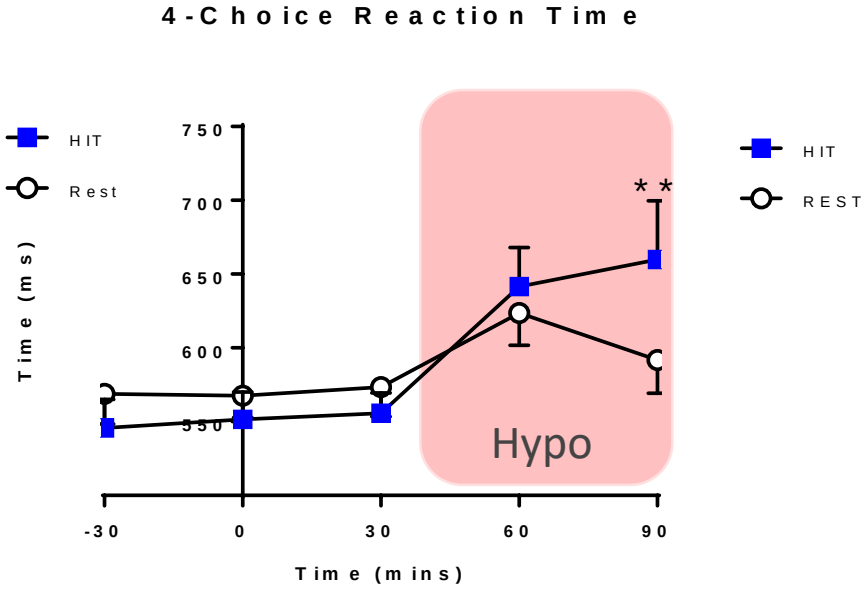
Hypoglycaemia Dishabituation with High Intensity Exercise: Type 1 diabetes with Impaired Hypoglycaemia Awareness

- Proof of concept, randomised, cross over study
- 12 participants:
  - 6 male, 6 female; Age 19-54 years
  - Type 1 diabetes
    - Median duration 24.5 years
    - HbA1c 56.5 (mmol/mol)
  - IAH (Gold score ≥ 4, Modified Clarke score ≥4, or DAFNE hypoglycaemia awareness rating 2 or 3)
- Single intervention; High Intensity Training (HIT) ***or*** Rest (Control)
- Hyperinsulinemic hypoglycemic clamp (90 minutes, 2.5 mmol/l) the following day.
- 2 week cooling off period
- Cross over to second arm of the study followed by matched hyperinsulinemic hypoglycemic clamp

Hypoglycaemia Dishabituation with High Intensity Exercise: Improves Counterregulatory Response during Subsequent Hypoglycaemia





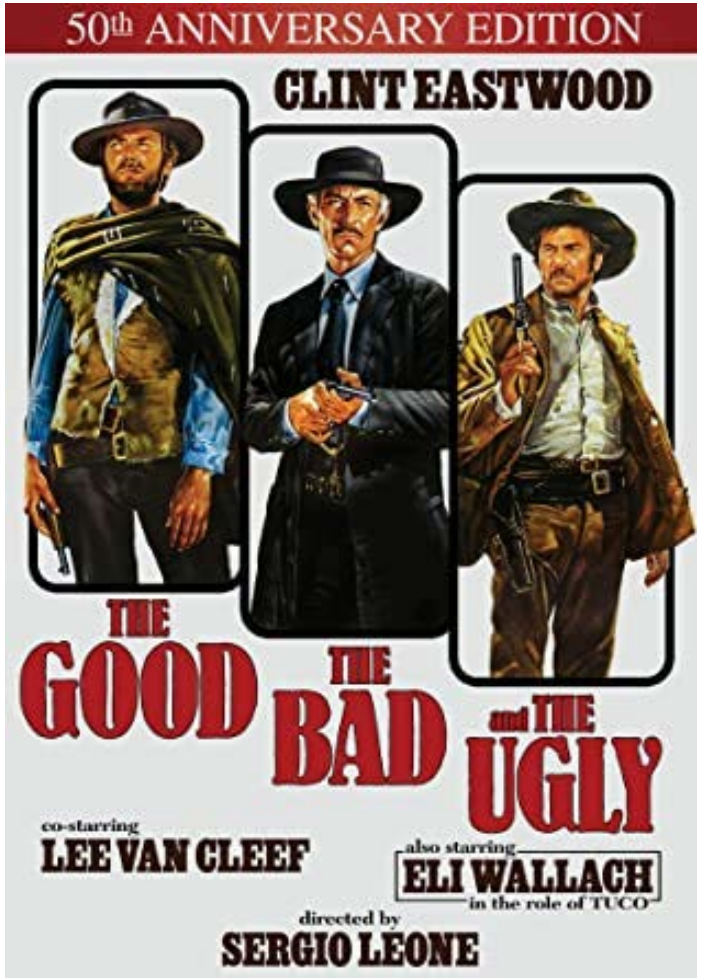


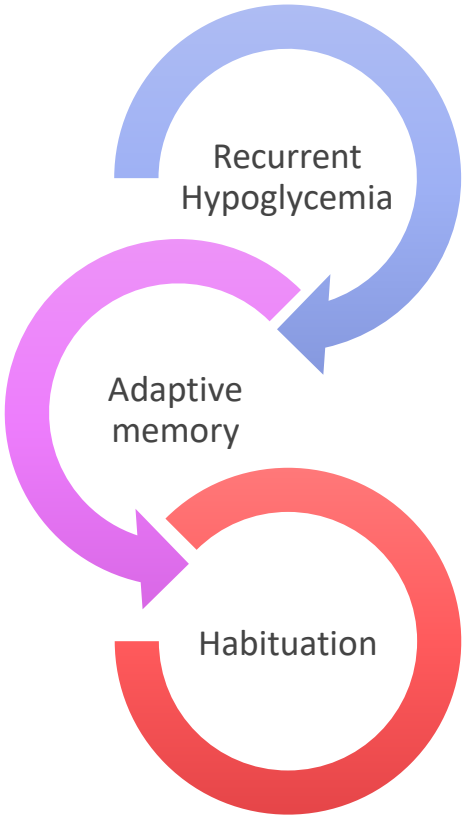
Diabetologia 2020 Apr;63(4):853-863.

Summary: Impaired Awareness of Hypoglycemia

- Hypoglycaemia
  - Sensing occurs within an integrated network of specialized glucose-sensing neurons
  - Sensing mechanisms show many similarities with pancreatic β- and α-cells
- Recurrent hypoglycaemia initiates an adaptive response in the brain the increases the threshold for counterregulatory hormonal and symptomatic responses as well as cognitive dysfunction
- Physiological, psychological and behavioral adaptations to recurrent hypoglycemia can be considered a specialized form of adaptive memory called Habituation
  - Dishabituation with HIT or cold exposure can at least temporarily improve hypoglycemia awareness
  - This may restore glutamatergic signaling in the hypothalamus

Habituation and Severe Hypoglycaemia

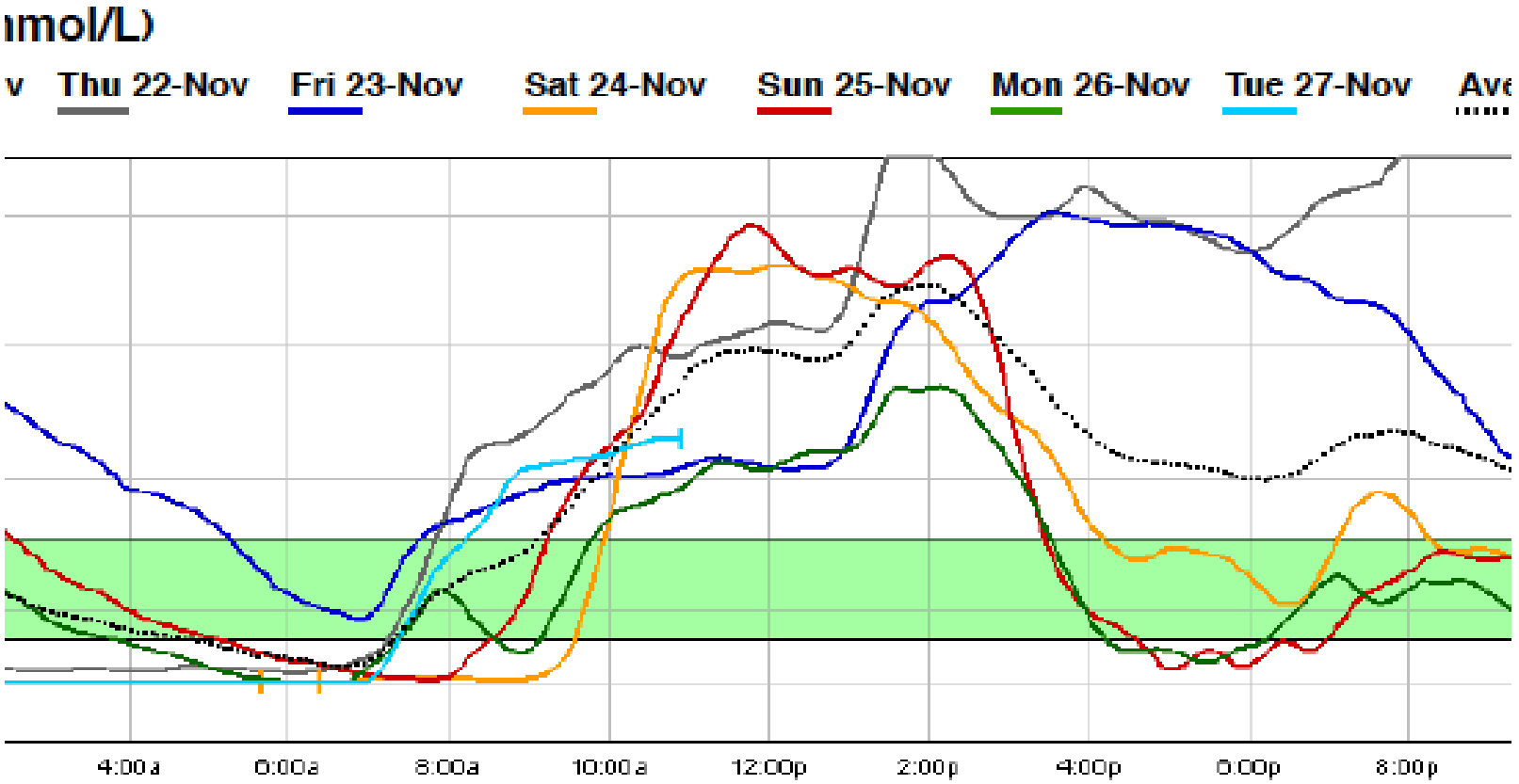




Maladaptive in Diabetes

- Hypoglycemia in T1D occurs in an unphysiological context
- Hyperinsulinemia and Hypoglucagonemia
- This overwhelms the adaptive response leading to severe hypoglycemia

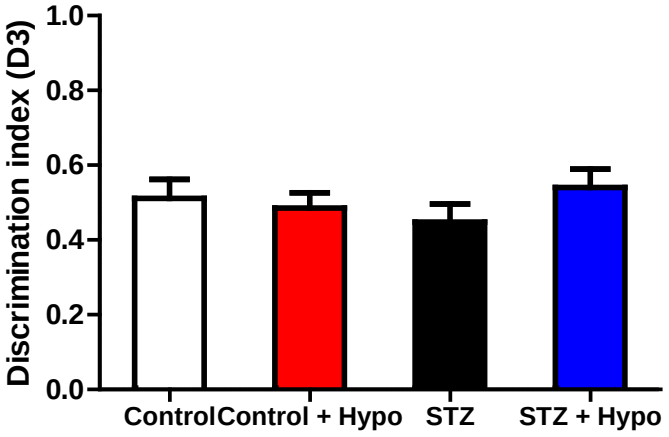
Hypoglycaemia: The Ugly



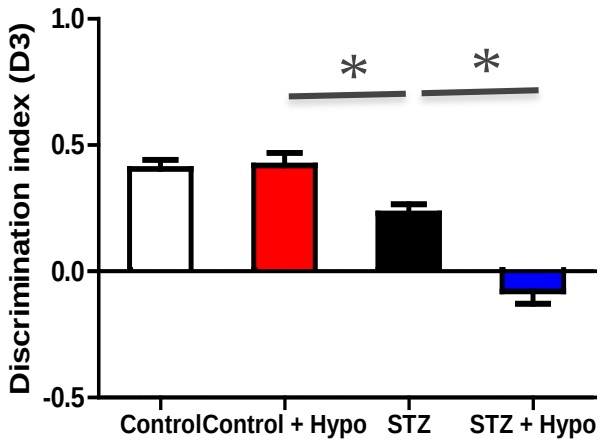
Cognitive dysfunction following recurrent hypoglycaemia in rodents withType 1 Diabetes

Discrimination index (D3)

Short-term Memory



Long-term Memory

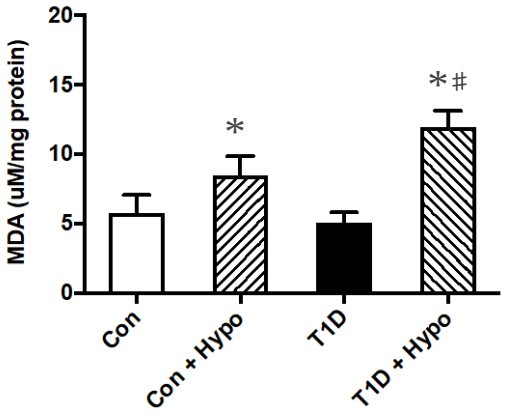


p<0.05 Control v STZ; # p<0.05 Control v hypo

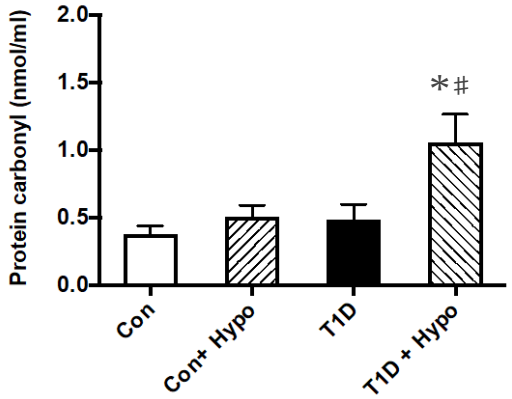
McNeilly AD et al 2016 Diabetes 65(10):3151-60

Recurrent Hypoglycaemia, Oxidative stress and Nrf2

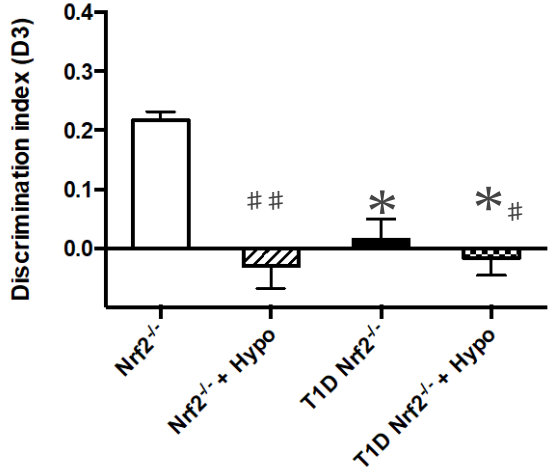
A) Lipid peroxidation



B) Protein carbonylation

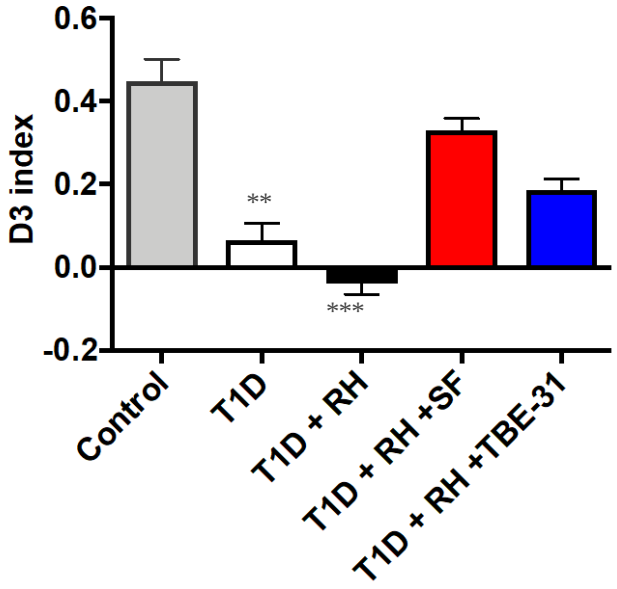


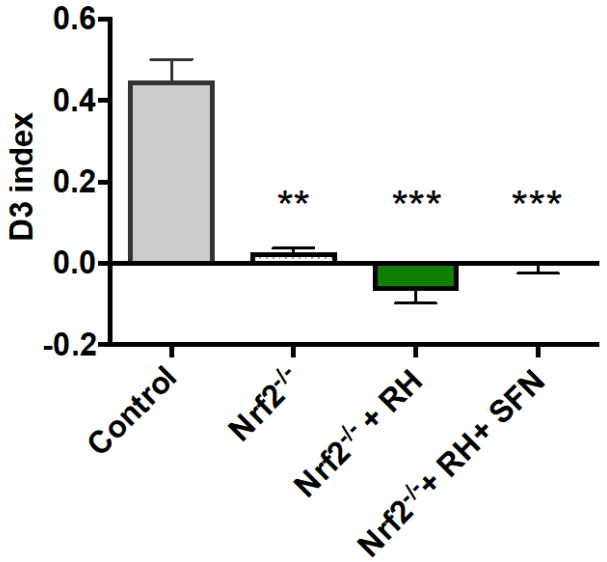
C) Nrf2 and cognition



McNeilly AD et al 2016 Diabetes 65(10):3151-60

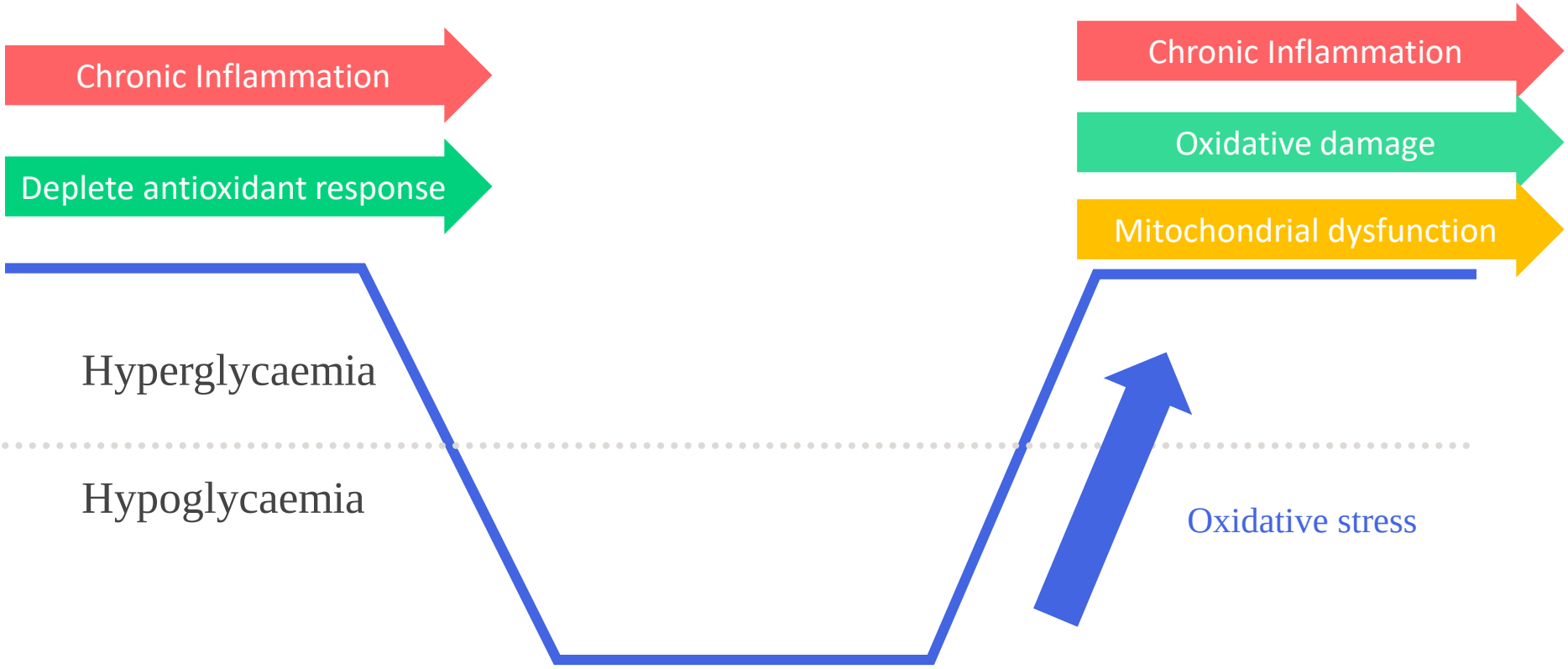
Long-term memory is restored by supplementation with Sulforaphane (SFN)





(n=8-10 per group; **p<0.01,***p<0.001)

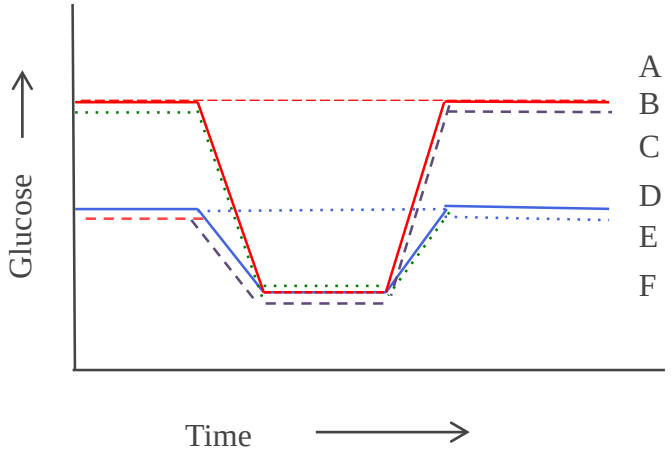
Hypo- and Hyper-Glycaemia;
The Scylla and Charybdis of Glucose Homeostasis In Type 1 Diabetes



Experimental design

- Male C57bl6 mice (8-10wks old)
- Control (non-diabetic) v T1D
- T1D (STZ 125mg/kg i.p) + linbit for 1 month
- Hyperinsulinaemic- Hypoglycaemic or Euglycaemic clamp
- Recovery to either Euglycaemia or Hyperglycaemia
- 16hrs recovery – tissue harvest for proteomics
- Proteomic analysis of hippocampal samples
- Validation by Western blot

Figure 1a Experimental design



A — Hyper – Hypo - Hyper

B — Hyper – Hyper - Hyper

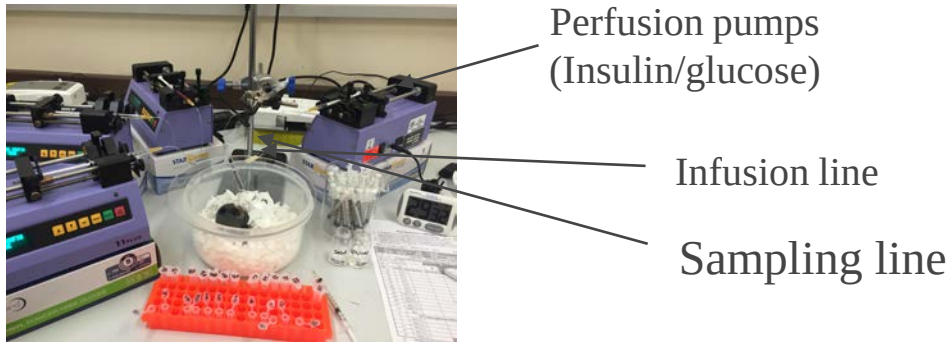
C — Hyper – Hypo - Eugly

D — Eugly - Eugly- Eugly

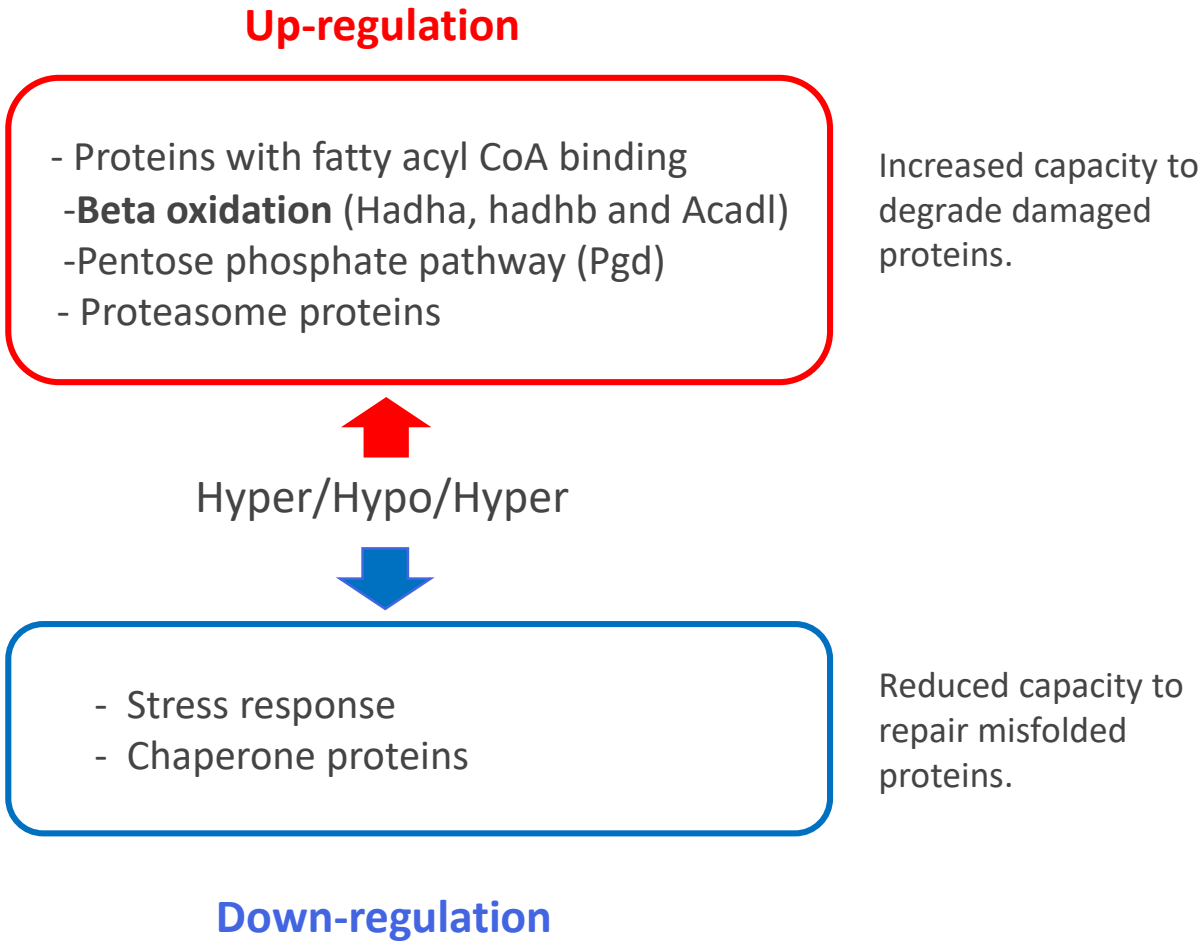
E — Eugly – Hypo - Eugly

F — Eugly – Hypo – Hyper

Figure 1b Hyperinsulinemic-hypoglycaemic clamp



SILAC proteomic analysis



Understanding Cardiovascular Damage Associated with Recurrent Hypoglycaemia

In vivo model

Male C57bl6J (20-25g)

STZ (150mg/kg i.p. in HBSS)

Linbit implant (Linshin 0.05U insulin/kg/day/SC)

Hypoglycaemia i.p. insulin (1-4 U/kg insulin i.p.; 3 episodes

• per week for 4 weeks).

Microvascular function

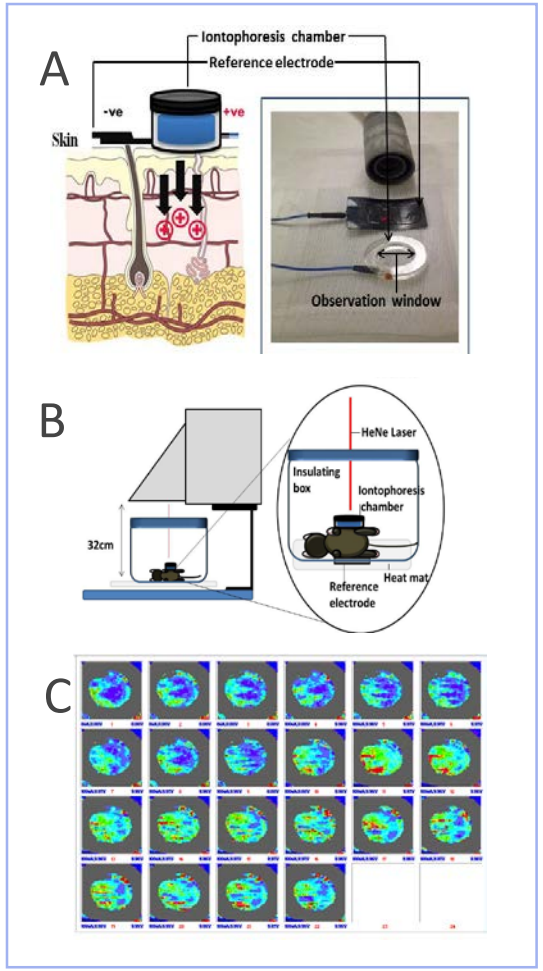
•laser Doppler Imaging (LDI) and iontophoresis

•Pre-treatment with L-NAME (20mg/kg i.p. 30 mins prior to LDI and iontophoresis) was used to assess the role of endothelium-derived nitric oxide (NO) in ACh-mediated vasodilation.

•Sodium nitroprusside (SNP) NO mediated, endothelium-independent vasodilation.

•Vasodilation in response to localised skin heating to 42^{o}C was assessed by LDI.

•Blood pressure was assessed using a non-invasive tail cuff occlusion method (Kent Scientific, UK



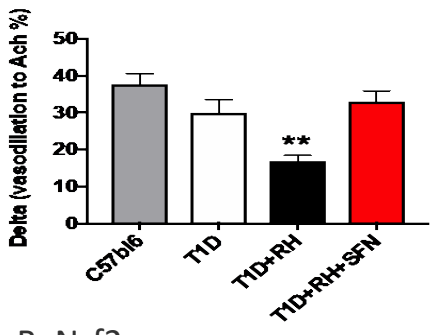
RH leads to endothelial dysfunction

Vasodilation in response to ACh and heat impaired in T1D and T1D+RH

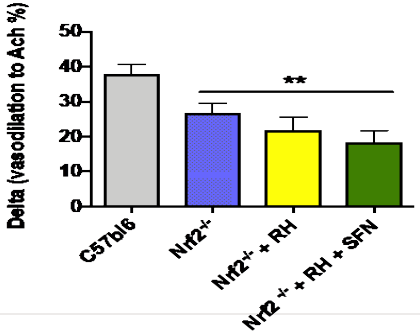
Response partially restored following treatment with SFN

Ach mediated vasodilation

A. T1D

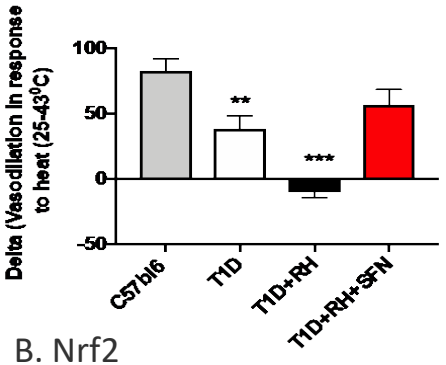


B. Nrf2

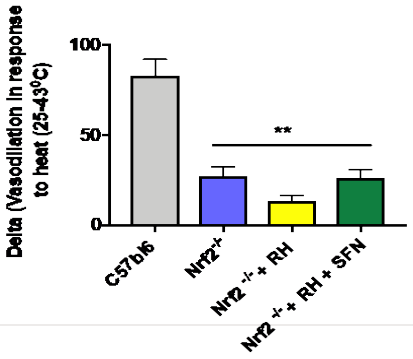


Max vasodilatory capacity

A. T1D

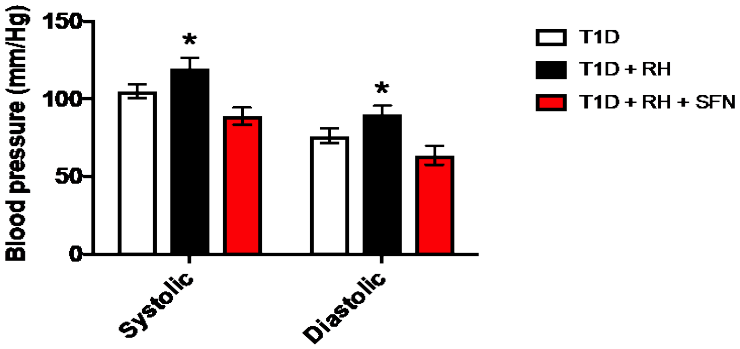


B. Nrf2

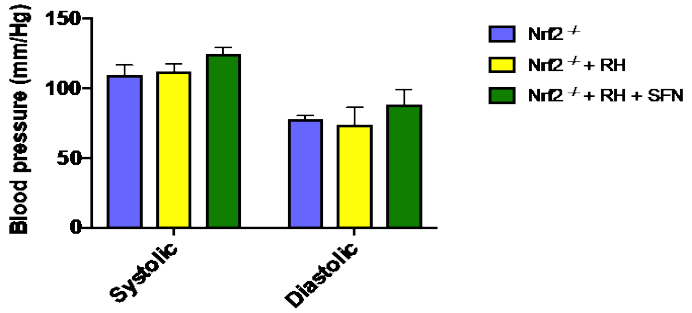


Blood pressure

A. T1D



B. Nrf2

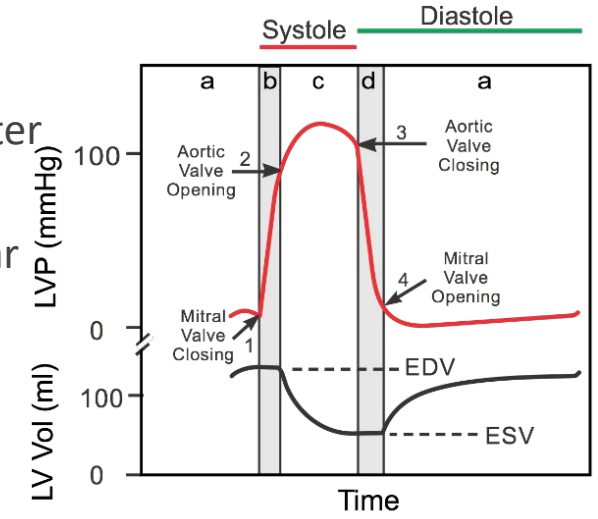


Mean ± SEM; n=8-10 per group; *p<0.05, **p<0.01, ***p<0.001 vs C57bl6/Nrf2 $^{-/-}$

Understanding Cardiovascular Damage Associated with Recurrent Hypoglycaemia

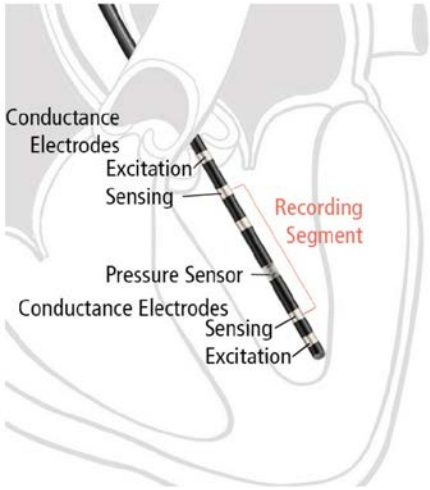
→Invasive measurement of cardiac cycle

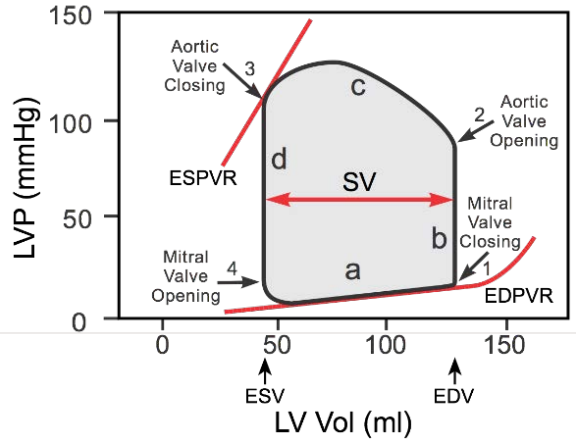
- → Catheterization of carotid artery with specialized PV catheter (Transonic)
- → Pressure and conductance sensors measure intraventricular pressure and blood volume throughout the cardiac cycle



→Collect Hemodynamic indictors of:

- → Cardiomyopathy
- → Ischemia
- → Valve disease
- → Arrhythmia
- → Compliance





Preliminary Conclusions

- Recurrent hypoglycaemia in diabetes may have consequences in a number of organs
  - Brain/ Heart / Renal
- Prior glycaemic control and glucose recovery post-hypoglycaemia influence the inflammatory and oxidative stress response to hypoglycaemia
- Future studies planned to examine this in more detail

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Simon Heller – Sheffield

Daniel West - Newcastle

Tim Jones - UWA

Paul Fournier- UWA

HypoRESOLVE

IHSG

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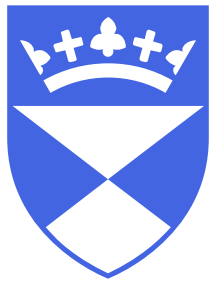

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