



University of
Zurich^{UZH}

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich



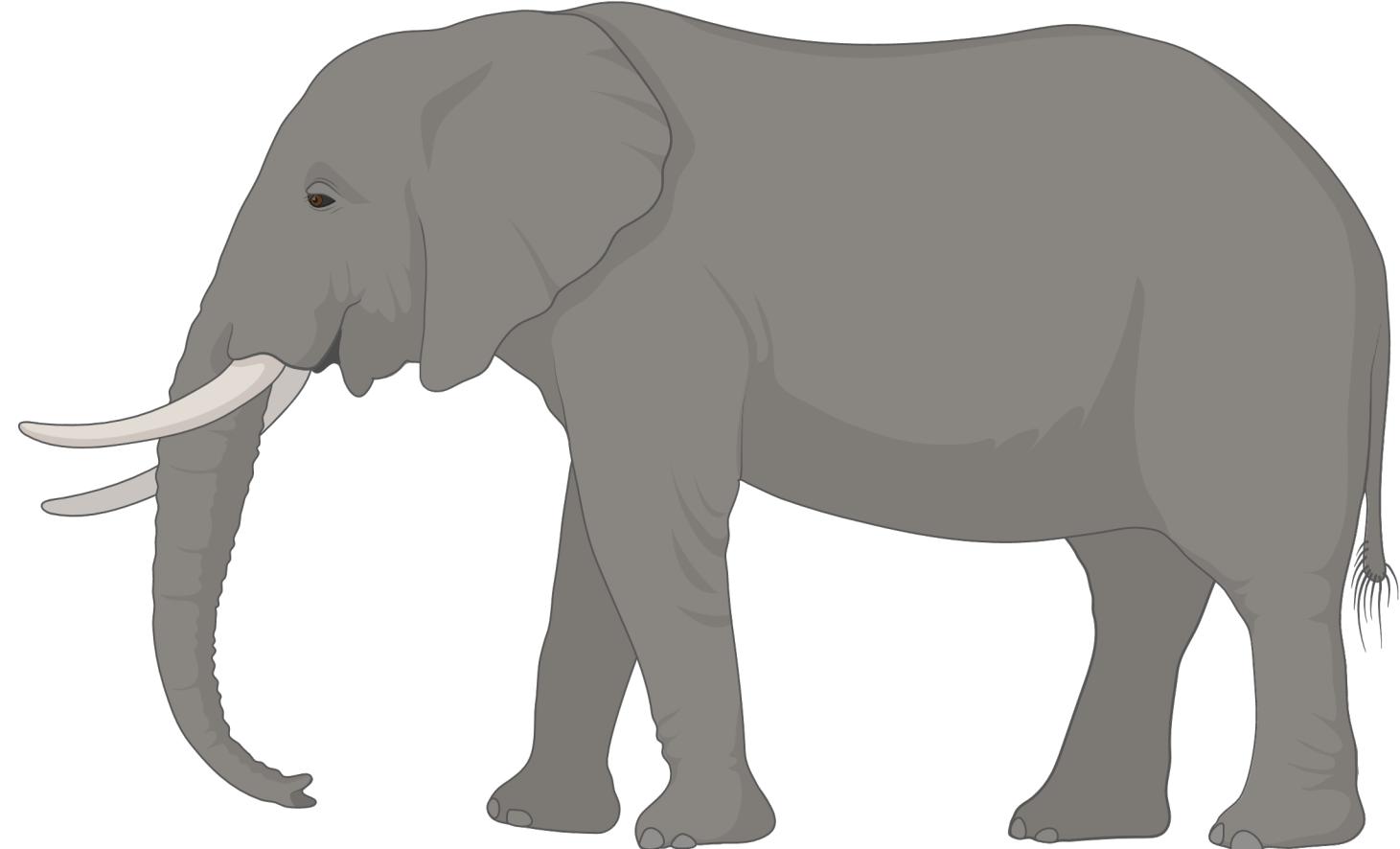
Translational Neuromodeling Unit

Fatigue

Inês Pereira

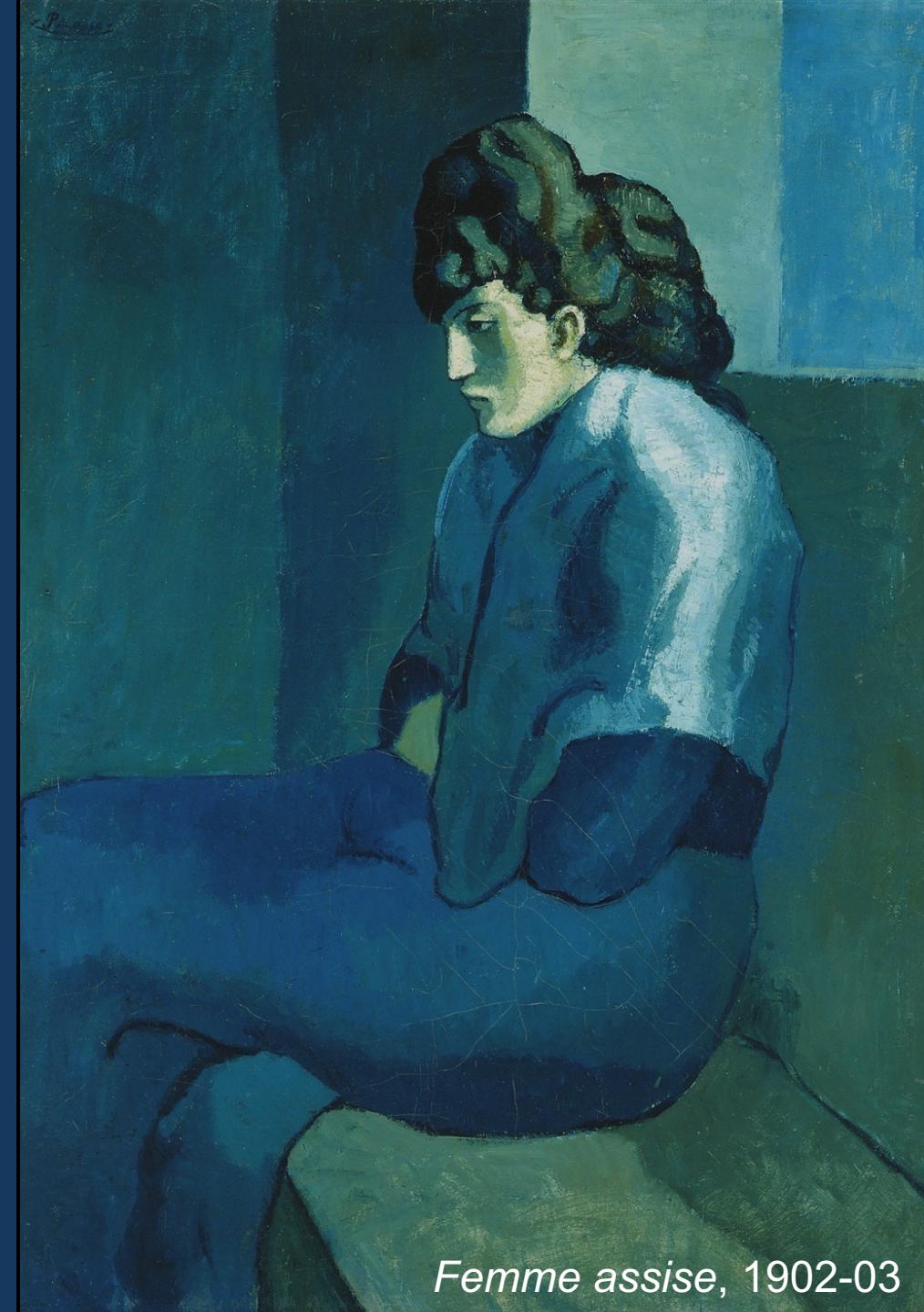
CPC Zurich 2022

Fatigue



Clinical case

- 48-year old
- Chief complaint:
 - Depressed mood
 - Low energy
 - Sleeping excessively
 - Lost interest in previous hobbies



Femme assise, 1902-03

Fatigue

Definition?

Fatigue

Definition?

Common problem?

Fatigue

Definition?

Common problem?

Fatigue

Causes?

Definition?

Common problem?

Fatigue

Causes?

Treatment?

Definitions

Is there one specific biomarker?

Is there one specific biomarker?

No.

A pathognomonic clinical sign, then?

A pathognomonic clinical sign, then?

No.

Based on the patient's subjective account?

Based on the patient's subjective account?

Yes, but...

What do you mean by fatigue?

Somnolence, sleepiness

What do you mean by fatigue?

Somnolence, sleepiness

What do you mean by fatigue?

Dyspnea on exertion

Somnolence, sleepiness

What do you mean by fatigue?

Muscle weakness

Dyspnea on exertion

Somnolence, sleepiness

Difficulty concentrating

What do you mean by fatigue?

Muscle weakness

Dyspnea on exertion

• • •

"a feeling arising from difficulty in initiation of or sustaining voluntary effort"

Chaudhuri and Behan, *Lancet*, 2004

"feeling that relates to the lack of motivation to deploy resources and engage in high effort performance to cope with their situation"

Dantzer et al., *Trends Neurosci*, 2014

"an overwhelming sense of tiredness that is out of proportion (in relation to the performed activity)"

Induruwa et al., *J Neurol Sci*, 2012

Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019

Subjective human experience of physical and mental weariness, sluggishness, and exhaustion.

Gelfand and Douglas, "Fatigue", *Harrison's principles of internal medicine*, 19th edition, 2018

Subjective human experience of physical and mental weariness, sluggishness, and exhaustion.

≠

Fatigability

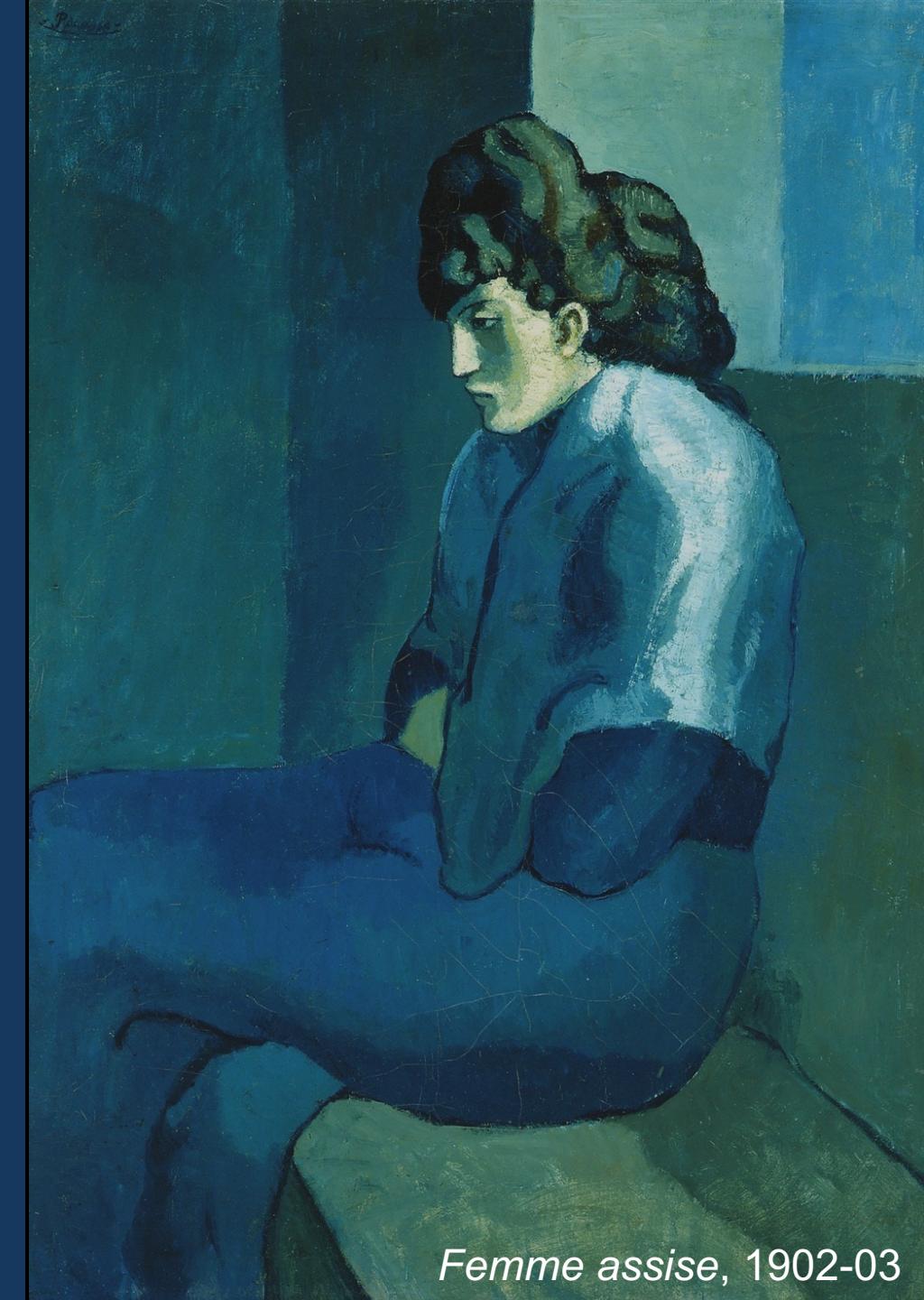
Gelfand and Douglas, "Fatigue", *Harrison's principles of internal medicine*, 19th edition, 2018

Kluger et al., *Neurology*, 2013

Penner and Paul, *Nature Reviews Neurology*, 2017

Depressed & fatigued

- 48-year old
- Chief complaint:
 - Depressed mood
 - Low energy
 - Sleeping excessively
 - Lost interest in previous hobbies



Femme assise, 1902-03

Epidemiology

Epidemiology

- General population (U.S.):
 - 6.7% point prevalence
 - 25% lifetime prevalence
- In specific conditions:
 - Multiple sclerosis: ~80% prevalence
 - Depression: fatigue is part of the diagnostic criteria
- Primary care setting (Europe and U.S.):
 - 21-33% of patients describe fatigue as an important problem
 - 10-25% of patients endorsed symptoms of subacute or chronic fatigue

Gelfand and Douglas, "Fatigue", *Harrison's principles of internal medicine*, 19th edition, 2018

<https://www.uptodate.com/contents/approach-to-the-adult-patient-with-fatigue>

DSM-5: Major depressive episode

- A. Five (or more) of the following symptoms have been present during the same two-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observations made by others (e.g., appears tearful).
 2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation)
 3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.
 4. Insomnia or hypersomnia nearly every day

DSM-5: Major depressive episode

5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
 6. Fatigue or loss of energy nearly every day
 7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
 8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by their subjective account or as observed by others)
 9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide
- [...]

ICD-10

In typical depressive episodes of all three varieties described below (mild (F32.0), moderate (F32.1), and severe (F32.2 and F32.3)), the individual usually suffers from depressed mood, loss of interest and enjoyment, and **reduced energy** leading to increased fatigability and diminished activity. Marked tiredness after only slight effort is common. Other common symptoms are:

- a) reduced concentration and attention;
- b) reduced self-esteem and self-confidence;
- c) ideas of guilt and unworthiness (even in a mild type of episode);
- d) bleak and pessimistic views of the future;
- e) ideas or acts of self-harm or suicide;
- f) disturbed sleep;
- g) diminished appetite.

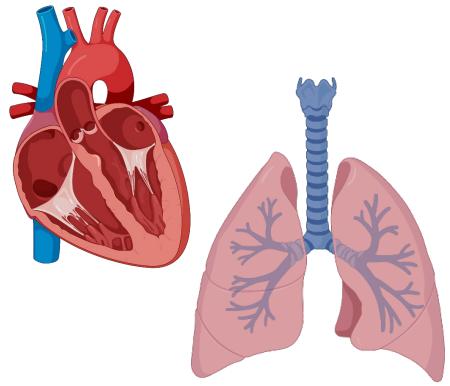
Causes of fatigue

Types of fatigue

- Acute (< 1 month)
- Subacute (1-6 months)
- Chronic (>6 months)

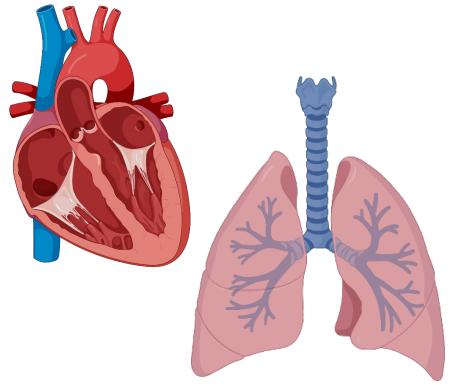
<https://www.uptodate.com/contents/approach-to-the-adult-patient-with-fatigue>

Fatigue

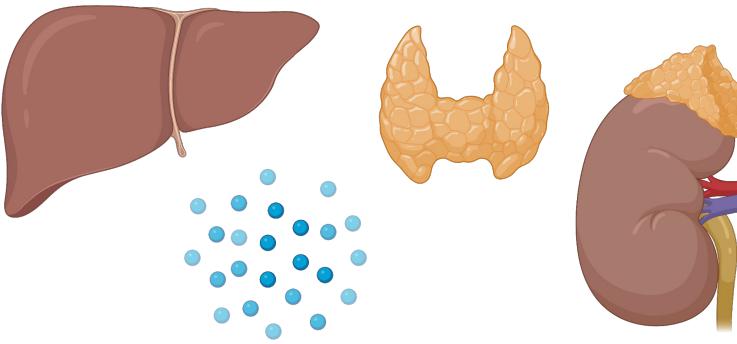


Cardiopulmonary

Fatigue

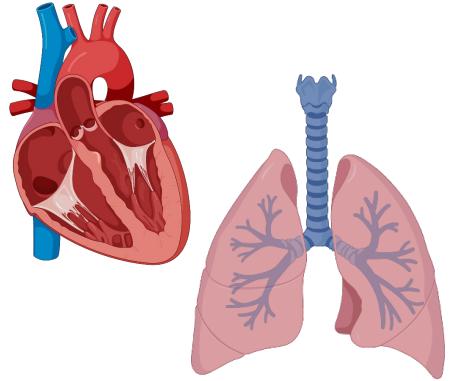


Cardiopulmonary

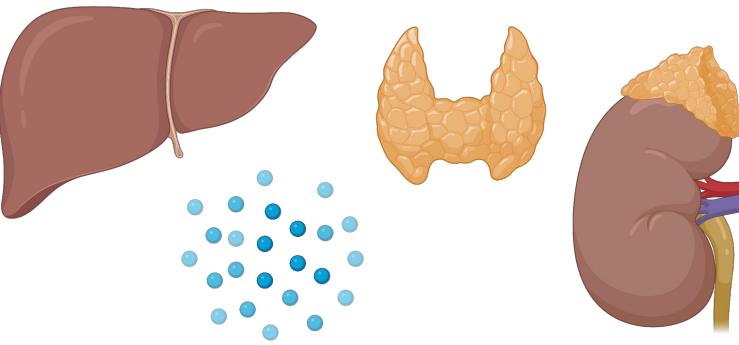


Endocrinologic/metabolic

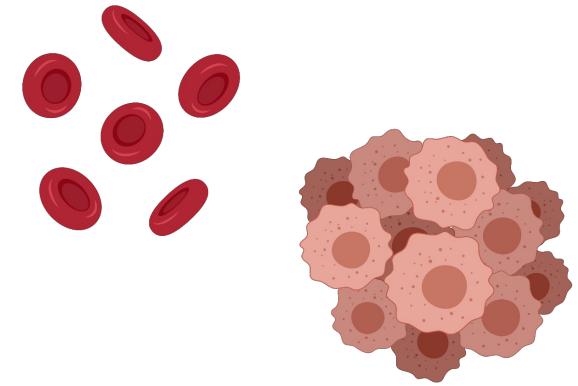
Fatigue



Cardiopulmonary

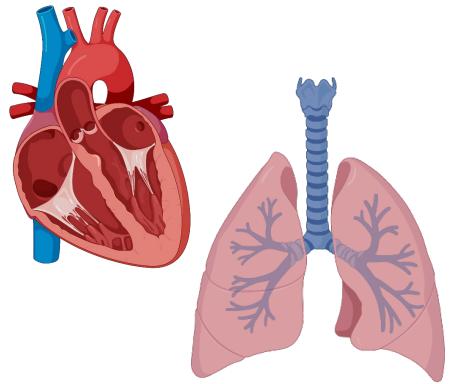


Endocrinologic/metabolic

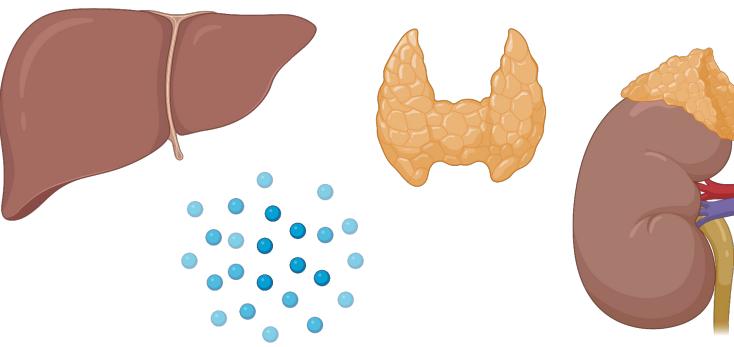


Hematologic/neoplastic

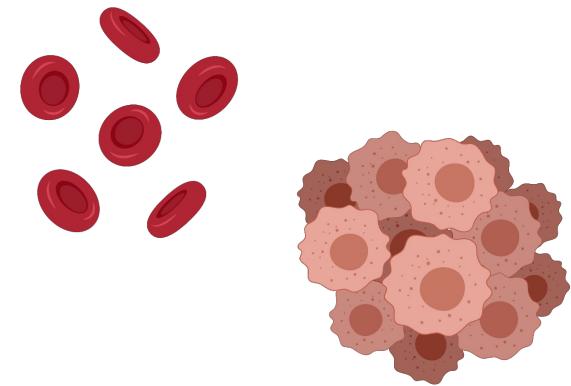
Fatigue



Cardiopulmonary

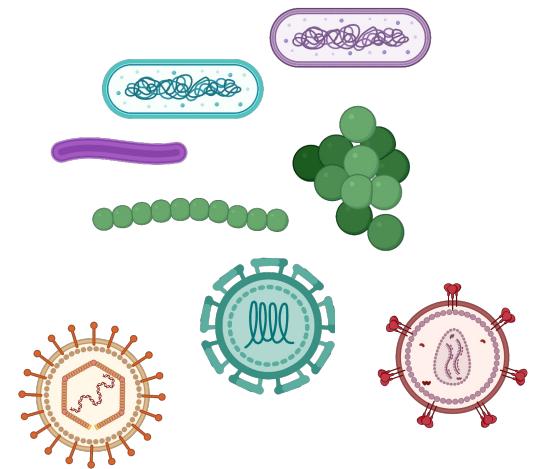


Endocrinologic/metabolic

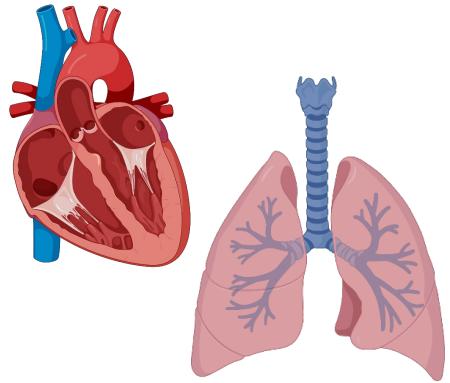


Hematologic/neoplastic

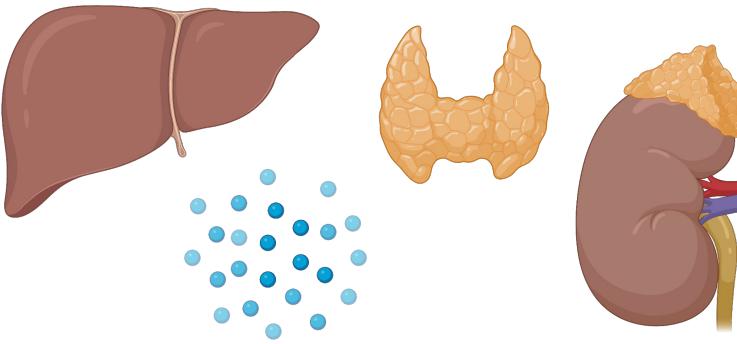
Fatigue



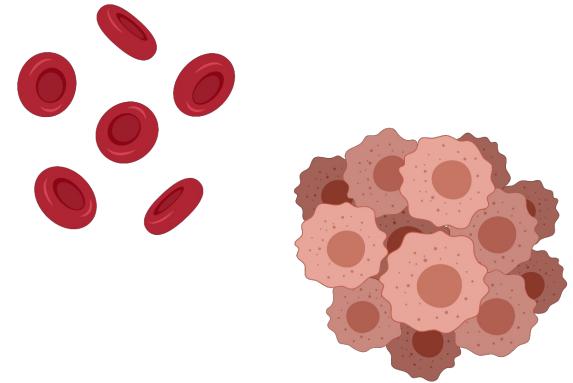
Infectious



Cardiopulmonary

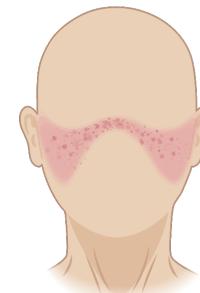
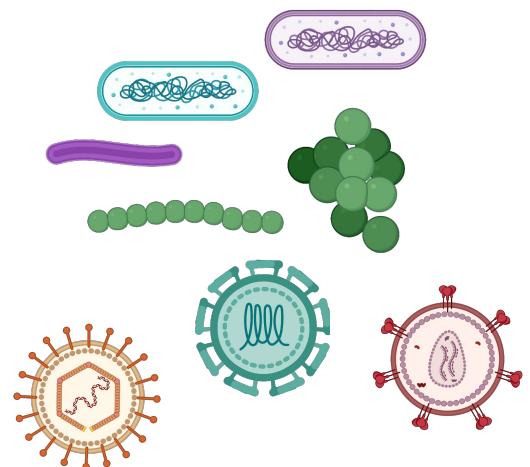


Endocrinologic/metabolic

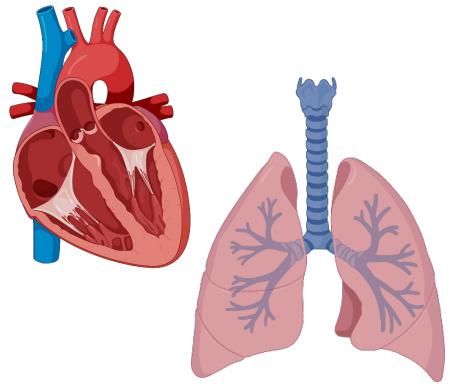


Hematologic/neoplastic

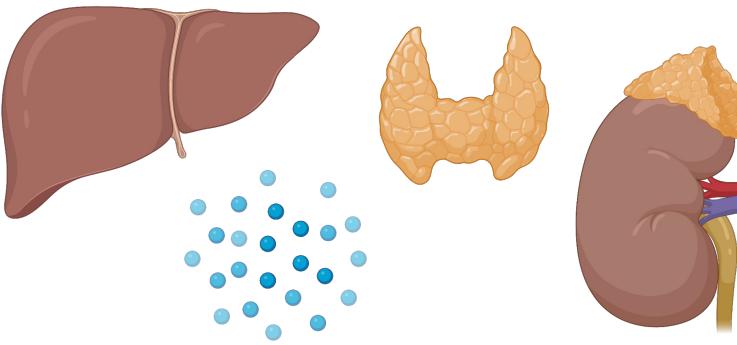
Fatigue



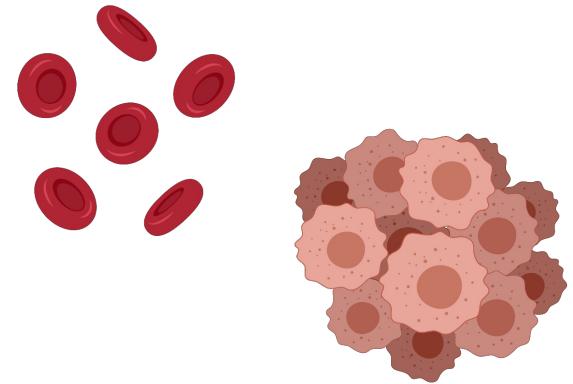
Rheumatologic



Cardiopulmonary



Endocrinologic/metabolic



Hematologic/neoplastic

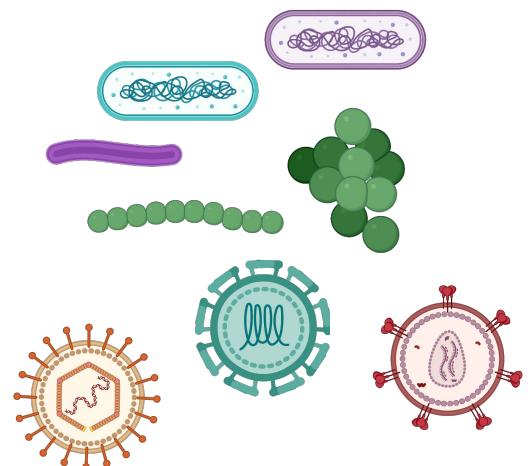
Fatigue



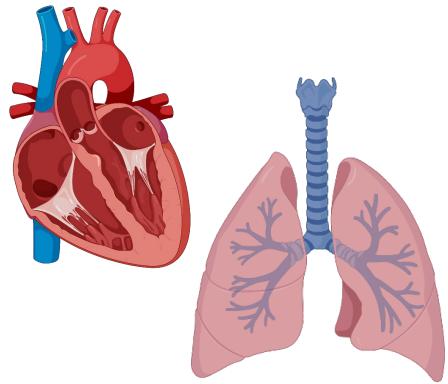
Psychiatric



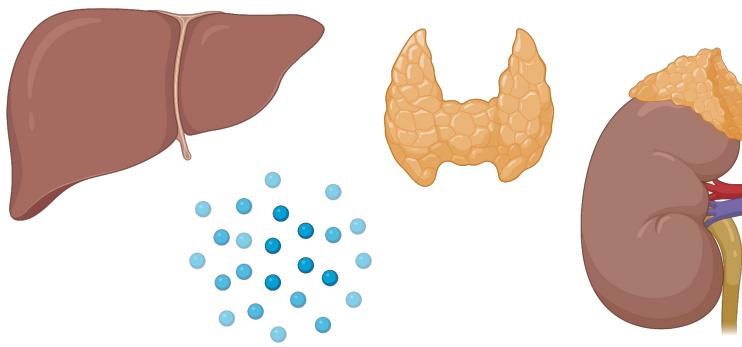
Rheumatologic



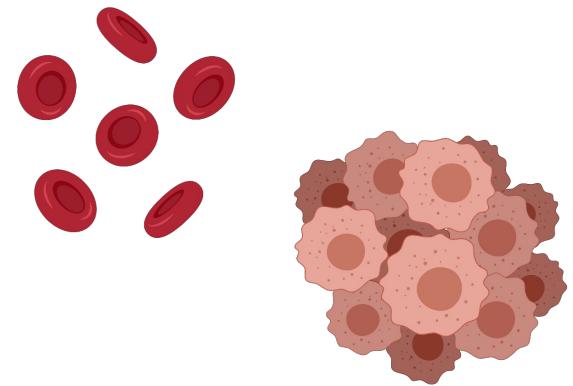
Infectious



Cardiopulmonary

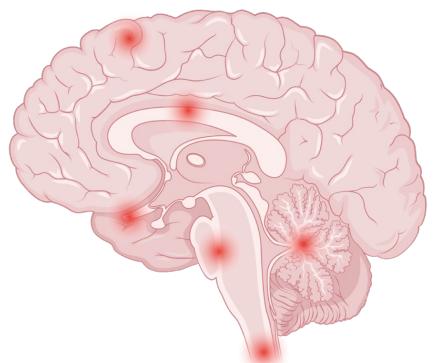


Endocrinologic/metabolic



Hematologic/neoplastic

Fatigue



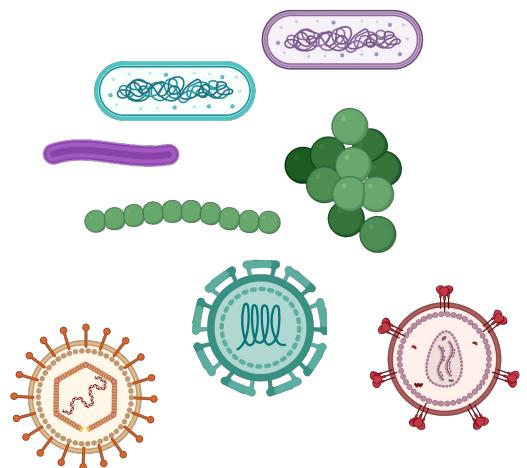
Neurologic



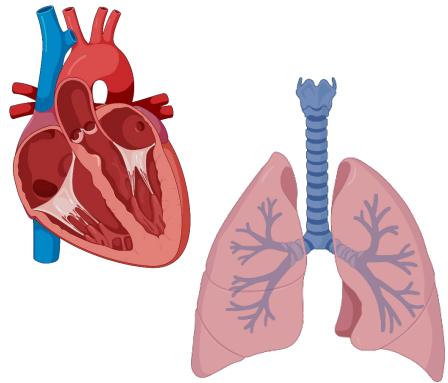
Psychiatric



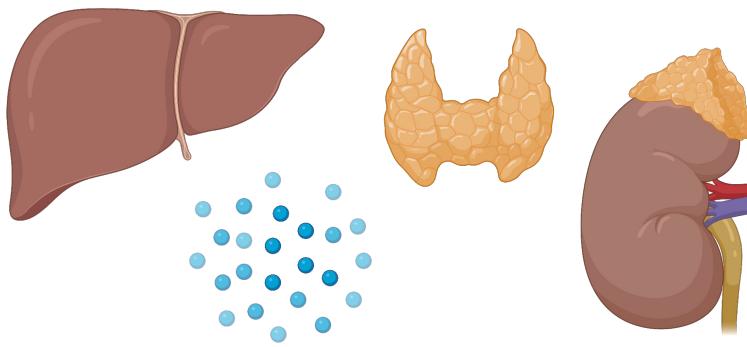
Rheumatologic



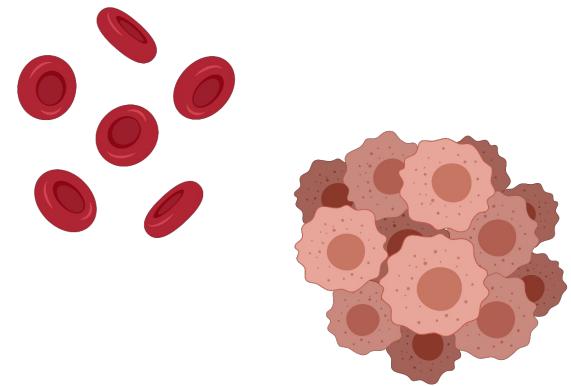
Infectious



Cardiopulmonary



Endocrinologic/metabolic

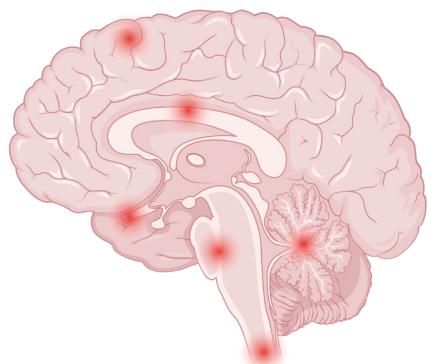


Hematologic/neoplastic

Fatigue



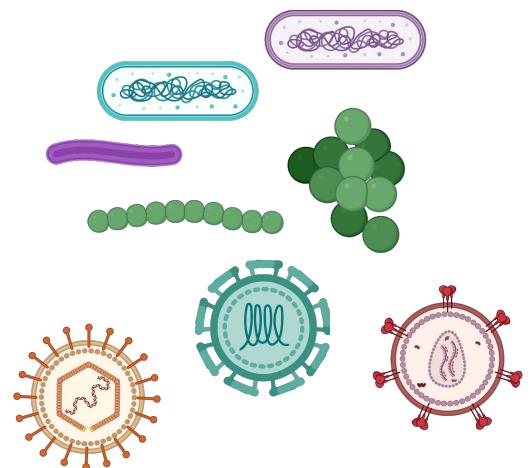
Medication



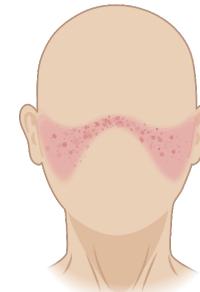
Neurologic



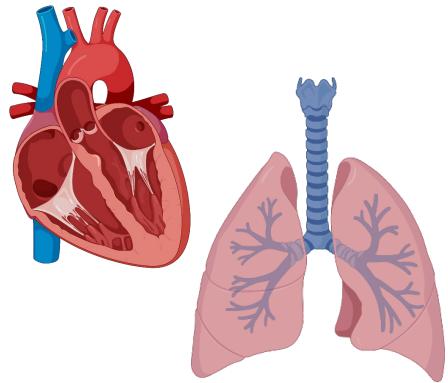
Psychiatric



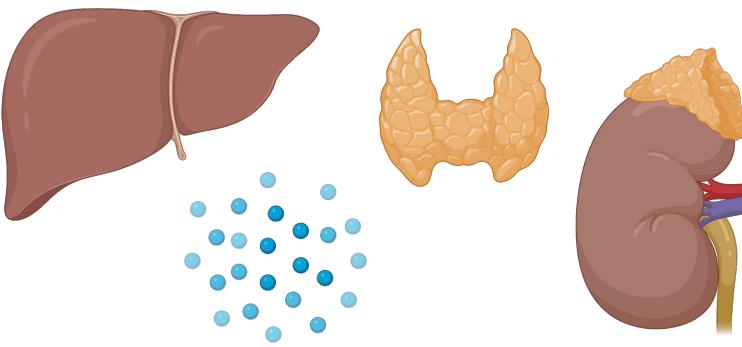
Infectious



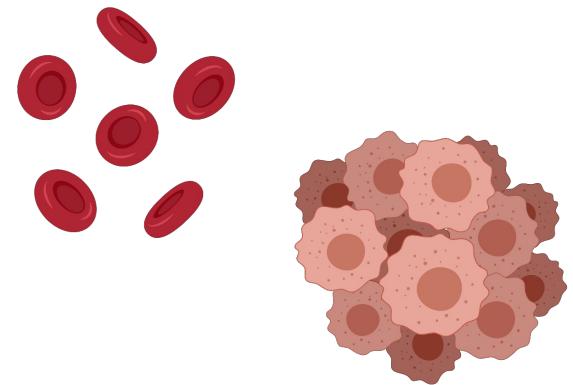
Rheumatologic



Cardiopulmonary



Endocrinologic/metabolic



Hematologic/neoplastic

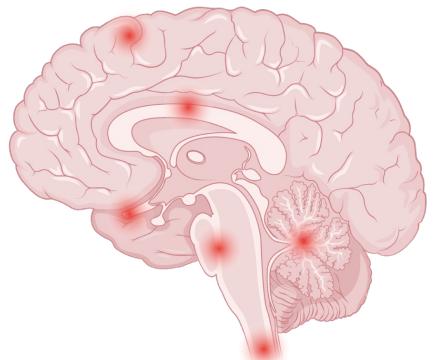


Substance use

Fatigue



Medication



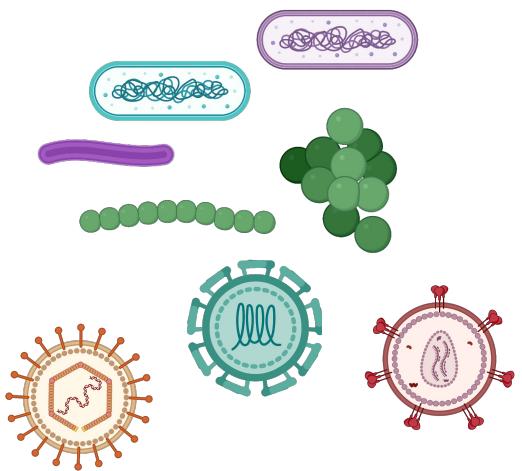
Neurologic



Psychiatric



Rheumatologic



Infectious

Approach to the patient with fatigue

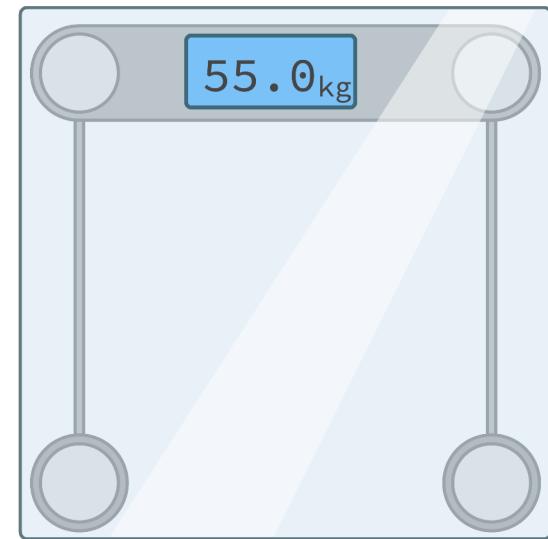
Approach to the patient with fatigue

- Medical history





>38 °C



Approach to the patient with fatigue

- Medical history



Fatigue Severity Scale (FSS, English version)*

	<i>strongly disagree</i>					<i>strongly agree</i>	
	1	2	3	4	5	6	7
1. My motivation is lower when I am fatigued.	0	0	0	0	0	0	0
2. Exercise brings on my fatigue.	0	0	0	0	0	0	0
3. I am easily fatigued.	0	0	0	0	0	0	0
4. Fatigue interferes with my physical functioning.	0	0	0	0	0	0	0
5. Fatigue causes frequent problems for me.	0	0	0	0	0	0	0
6. My fatigue prevents sustained physical functioning.	0	0	0	0	0	0	0
7. Fatigue interferes with carrying out certain duties and responsibilities.	0	0	0	0	0	0	0
8. Fatigue is among my three most disabling symptoms.	0	0	0	0	0	0	0
9. Fatigue interferes with my work, family, or social life.	0	0	0	0	0	0	0

**Patients are instructed to choose a number from 1 to 7 that indicates their degree of agreement with each statement where 1 indicates strongly disagree and 7, strongly agree. [Krupp et al, Arch Neurol 1989]*

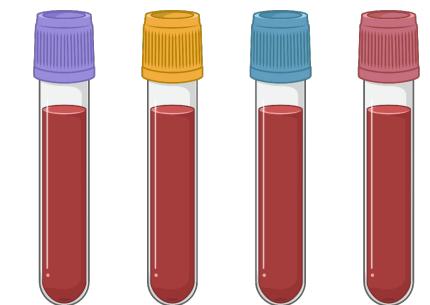
Approach to the patient with fatigue

- Medical history
- Physical examination



Approach to the patient with fatigue

- Medical history
- Physical examination
- Laboratory and radiological studies



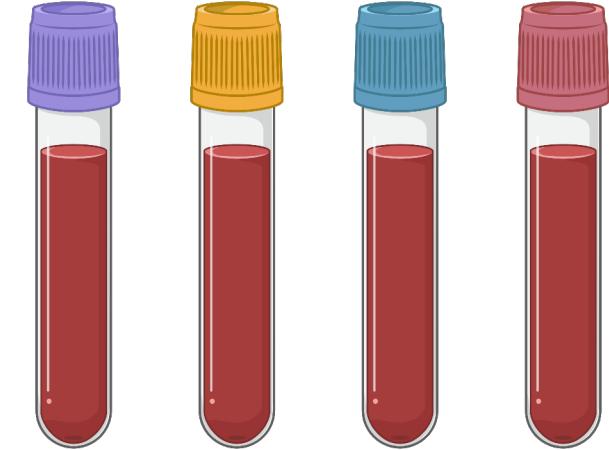
Laboratory and radiological studies

- Initial laboratory testing
 - Complete blood count with differential count



Laboratory and radiological studies

- Initial laboratory testing
 - Complete blood count with differential count
 - Chemistries (including glucose, electrolytes, renal and hepatic function tests)

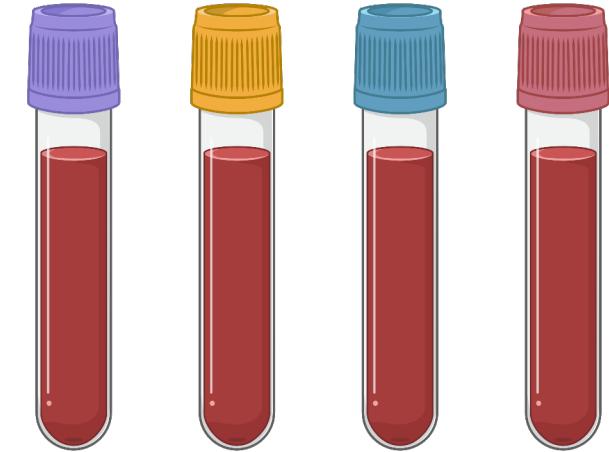


Gelfand and Douglas, "Fatigue", *Harrison's principles of internal medicine*, 19th edition, 2018

<https://www.uptodate.com/contents/approach-to-the-adult-patient-with-fatigue>

Laboratory and radiological studies

- Initial laboratory testing
 - Complete blood count with differential count
 - Chemistries (including glucose, electrolytes, renal and hepatic function tests)
 - Thyroid-stimulating hormone

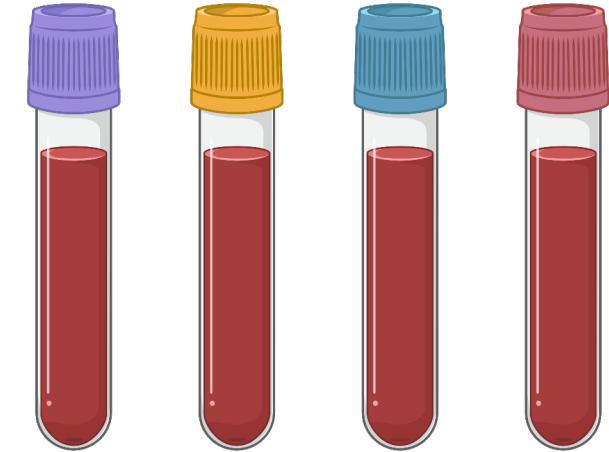


Gelfand and Douglas, "Fatigue", *Harrison's principles of internal medicine*, 19th edition, 2018

<https://www.uptodate.com/contents/approach-to-the-adult-patient-with-fatigue>

Laboratory and radiological studies

- Initial laboratory testing
 - Complete blood count with differential count
 - Chemistries (including glucose, electrolytes, renal and hepatic function tests)
 - Thyroid-stimulating hormone
 - Serologic testing dependent on risk factors (e.g., hepatitis C virus, HIV)

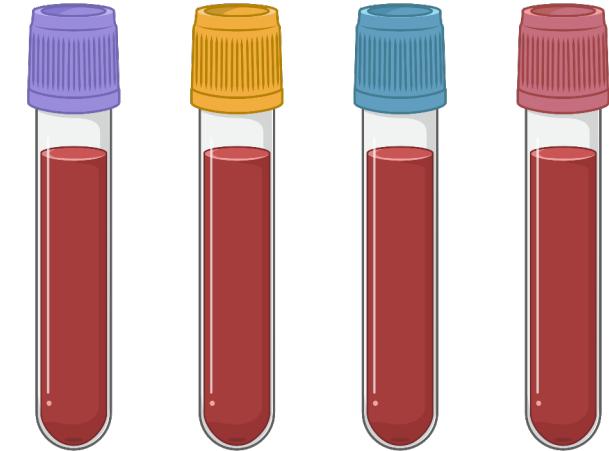


Gelfand and Douglas, "Fatigue", *Harrison's principles of internal medicine*, 19th edition, 2018

<https://www.uptodate.com/contents/approach-to-the-adult-patient-with-fatigue>

Laboratory and radiological studies

- Initial laboratory testing
 - Complete blood count with differential count
 - Chemistries (including glucose, electrolytes, renal and hepatic function tests)
 - Thyroid-stimulating hormone
 - Serologic testing dependent on risk factors (e.g., hepatitis C virus, HIV)
 - [...]

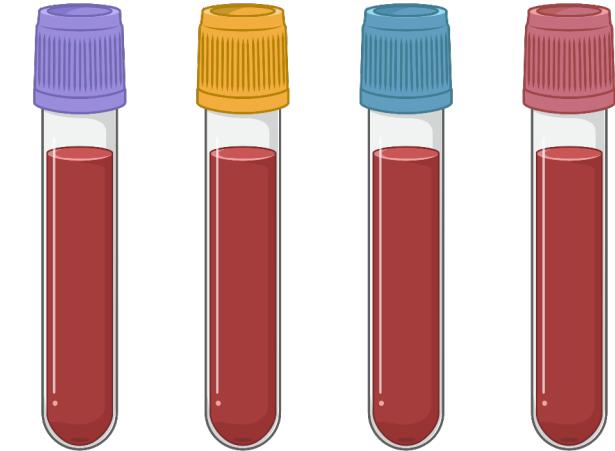


Gelfand and Douglas, "Fatigue", *Harrison's principles of internal medicine*, 19th edition, 2018

<https://www.uptodate.com/contents/approach-to-the-adult-patient-with-fatigue>

Laboratory and radiological studies

- Initial laboratory testing
 - Complete blood count with differential count
 - Chemistries (including glucose, electrolytes, renal and hepatic function tests)
 - Thyroid-stimulating hormone
 - Serologic testing dependent on risk factors (e.g., hepatitis C virus, HIV)
 - [...]
- Updating of cancer screening interventions



Gelfand and Douglas, "Fatigue", *Harrison's principles of internal medicine*, 19th edition, 2018

<https://www.uptodate.com/contents/approach-to-the-adult-patient-with-fatigue>

Management

Management

- Establish therapeutic goals



Management

- Establish therapeutic goals
- Treat underlying condition(s)



Management

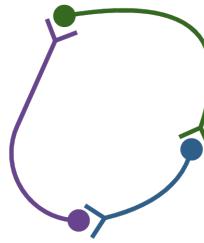
- Establish therapeutic goals
- Treat underlying condition(s)
- Address residual or idiopathic fatigue
 - Pharmacological interventions
 - Non-pharmacological interventions
 - Cognitive behavioral therapy
 - Exercise therapy
 - Occupational therapy



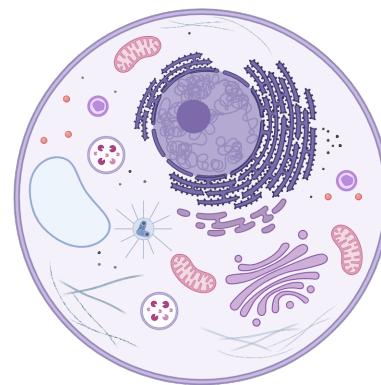
Pathophysiology of fatigue

???

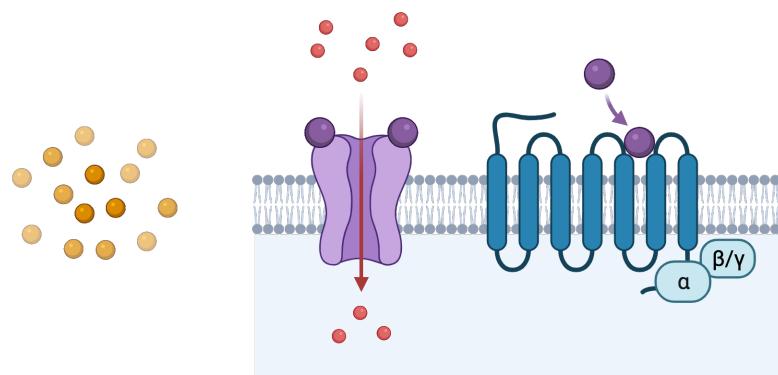
Circuit level



Cellular level



Molecular level



IL-6

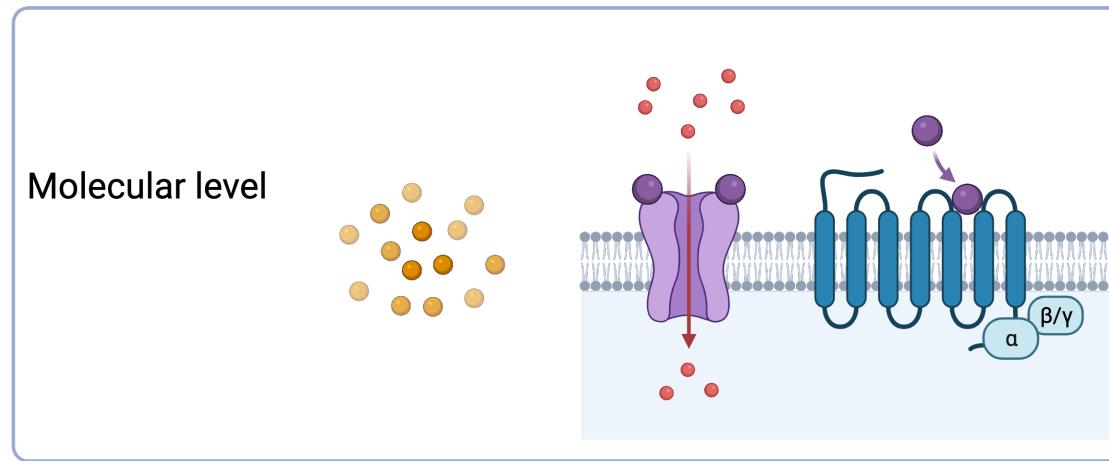
IL-1

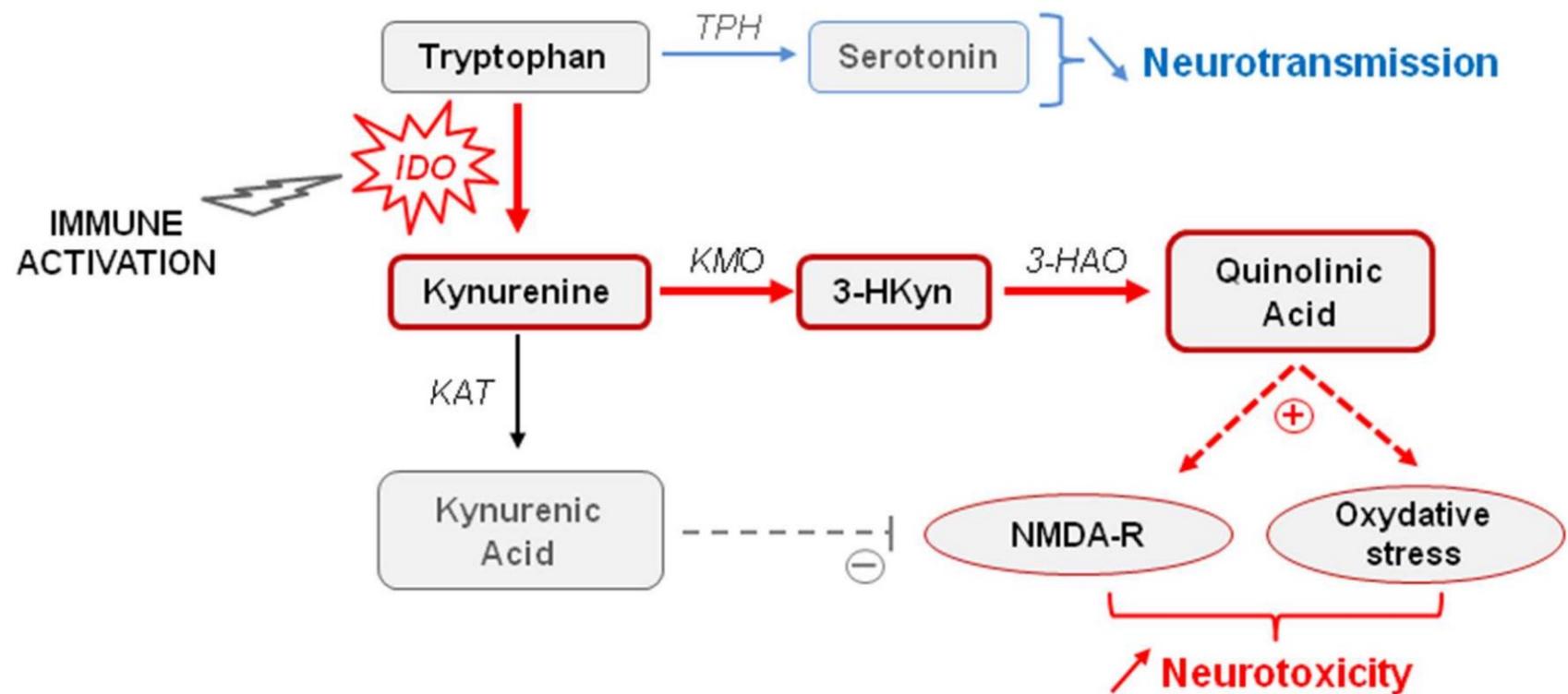
TNF- α

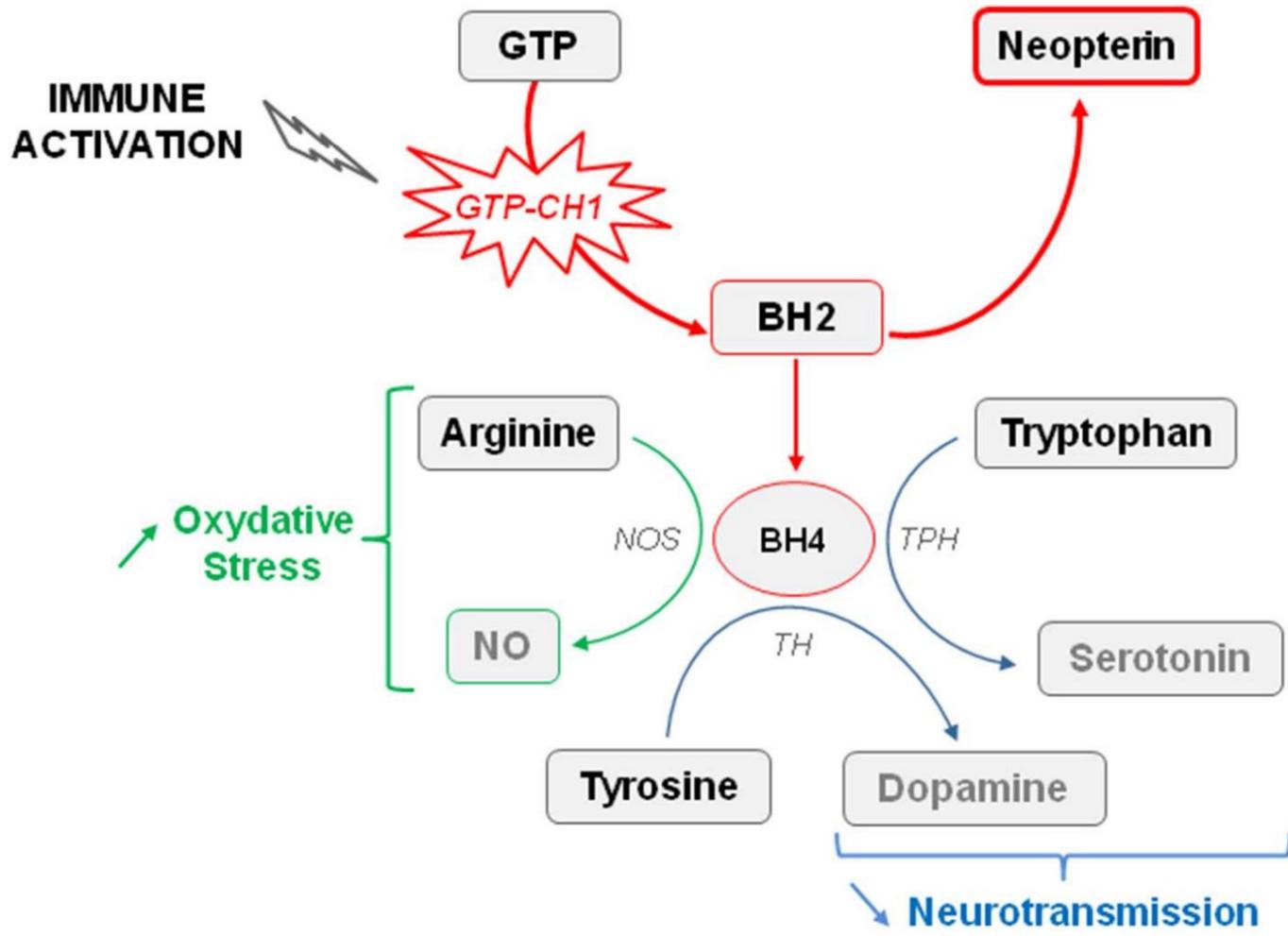
CRP

INF- α

INF- γ







Allostatic Self-efficacy: A Metacognitive Theory of Dyshomeostasis-Induced Fatigue and Depression

Klaas E. Stephan^{1, 2, 3}, Zina M. Manjaly^{1, 4}, Christoph D. Mathys², Lilian A. E. Weber¹, Saeed Paliwal¹, Tim Gard^{1, 5}, Marc Tittgemeyer³, Stephen M. Fleming², Helene Haker¹, Anil K. Seth⁶ and Frederike H. Petzschner¹*

¹ Translational Neuromodeling Unit, Institute for Biomedical Engineering, University of Zurich and ETH Zurich, Zurich, Switzerland, ² Wellcome Trust Centre for Neuroimaging, University College London, London, UK, ³ Max Planck Institute for Metabolism Research, Cologne, Germany, ⁴ Department of Neurology, Schulthess Clinic, Zurich, Switzerland, ⁵ Center for Complementary and Integrative Medicine, University Hospital Zurich, Zurich, Switzerland, ⁶ Sackler Centre for Consciousness Science, School of Engineering and Informatics, University of Sussex, Brighton, UK

Homeostasis

[The] maintenance of nearly constant conditions in the internal [bodily] environment

Walter Cannon, 1929

Homeostasis

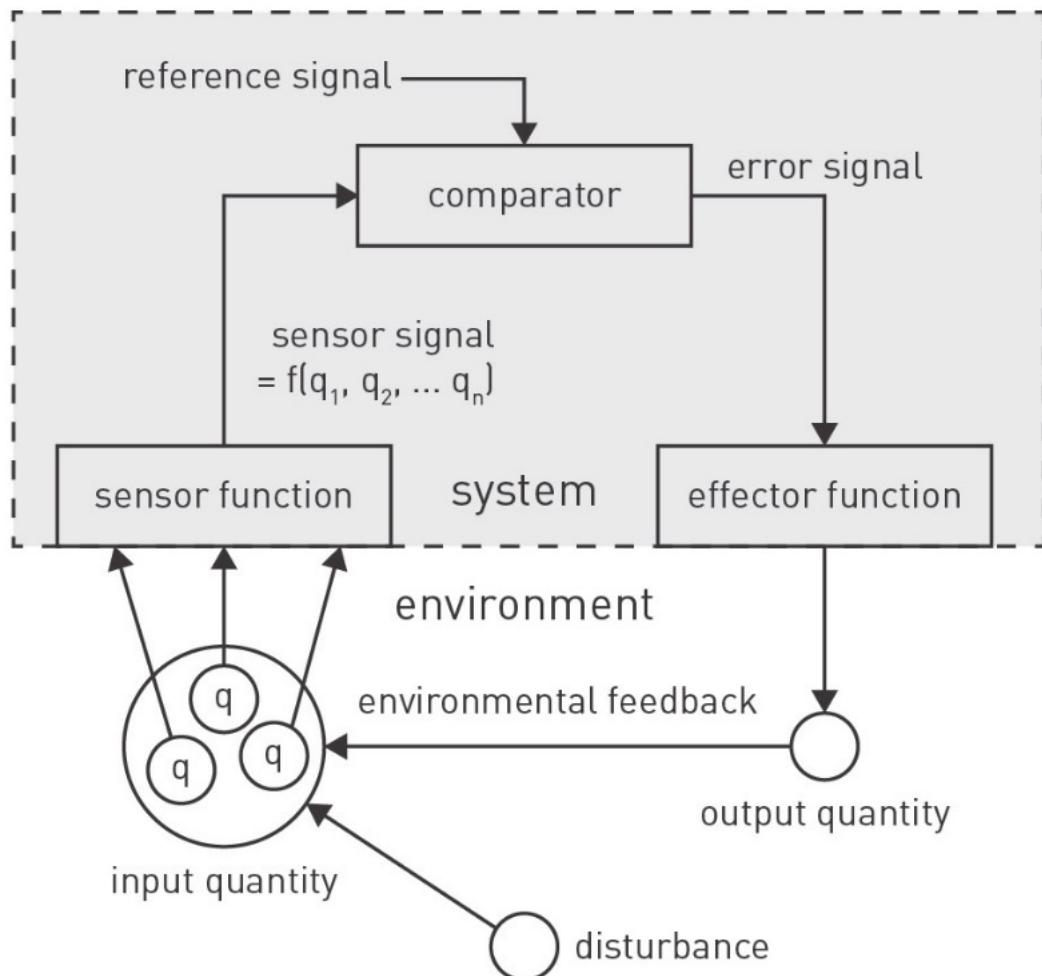


Figure from: Powers, *Science*, 1973

Based on slide from Klaas Enno Stephan

Homeostasis

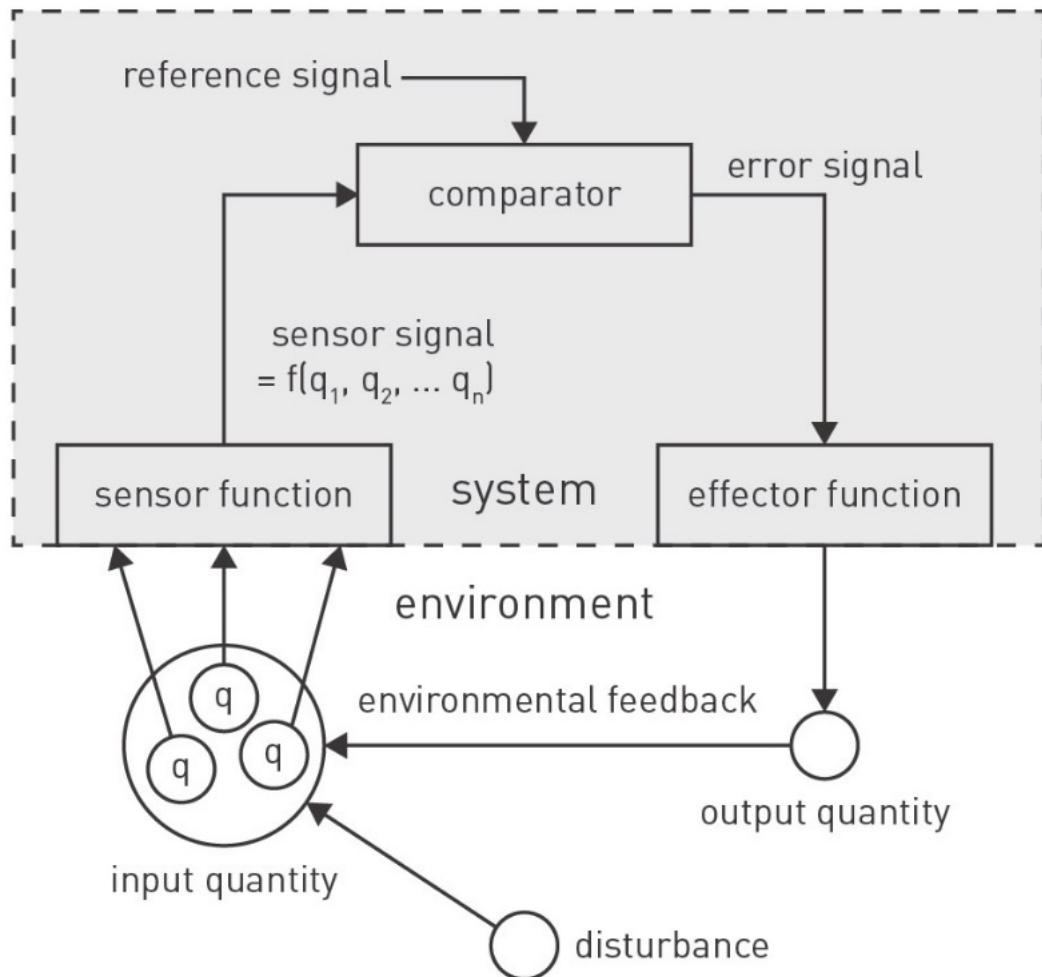


Figure from: Powers, *Science*, 1973

Allostasis

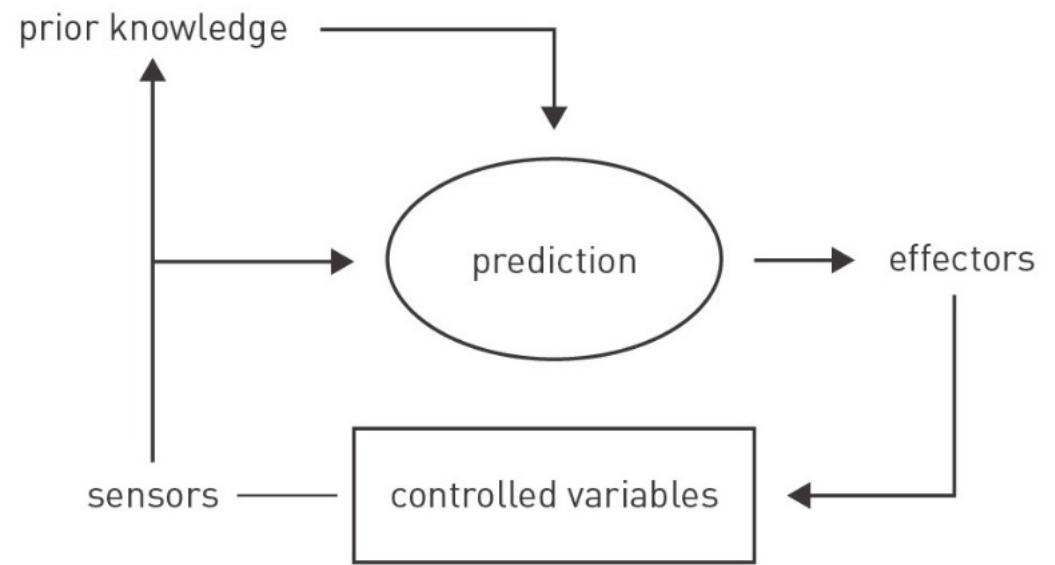
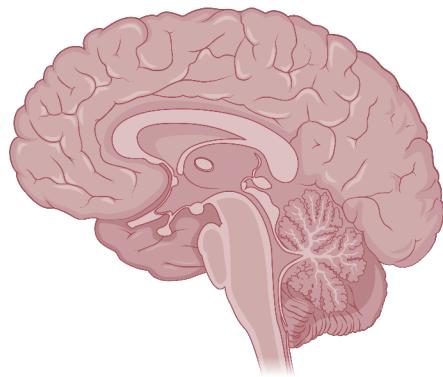


Figure from: Sterling, *Physiol. Behav.*, 2012

Based on slide from Klaas Enno Stephan



Based on figure from Stephan et al., *Frontiers in Human Neuroscience*, 2016

$$p(x|y) = \frac{p(y|x)p(x)}{p(y)}$$

prior

$$p(x|y) = \frac{p(y|x)p(x)}{p(y)}$$

$$p(x|y) = \frac{p(y|x)p(x)}{p(y)}$$

likelihood

prior

$$p(x|y) = \frac{likelihood \cdot prior}{posterior}$$
$$p(x|y) = \frac{p(y|x)p(x)}{p(y)}$$

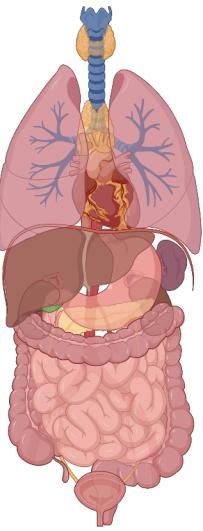
$$p(x|y, m) = \frac{\text{likelihood} \quad \text{prior}}{p(y|m)}$$

posterior

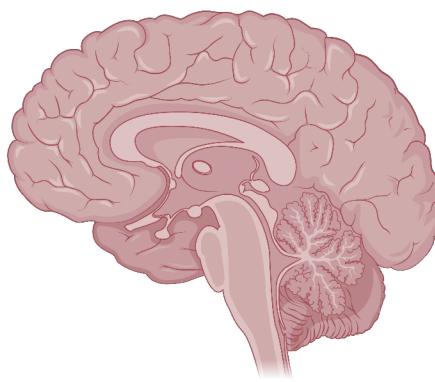
$$p(x|y, m) = \frac{\text{likelihood} \quad \text{prior}}{\text{posterior} \quad \text{model evidence}}$$
$$p(x|y, m) = \frac{p(y|x, m)p(x|m)}{p(y|m)}$$

$$p(x|y, m) = \frac{\text{likelihood} \quad \text{prior}}{\text{posterior} \quad \text{model evidence}}$$
$$= \frac{p(y|x, m)p(x|m)}{p(y|m)}$$

$$\log p(y|m) = -S(y|m)$$



$$\frac{p(x|y, m)}{p(y|x, m)p(x|m)}$$



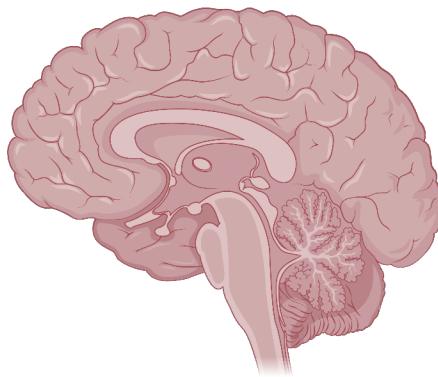
$$\frac{p(x|y, m)}{p(y|x, m)p(x|m)}$$



Based on figure from Stephan et al., *Frontiers in Human Neuroscience*, 2016



$$\frac{p(x|y, m)}{p(y|x, m)p(x|m)}$$



$$\frac{p(x|y, m)}{p(y|x, m)p(x|m)}$$



Interoceptive surprise

Exteroceptive surprise

Based on figure from Stephan et al., *Frontiers in Human Neuroscience*, 2016

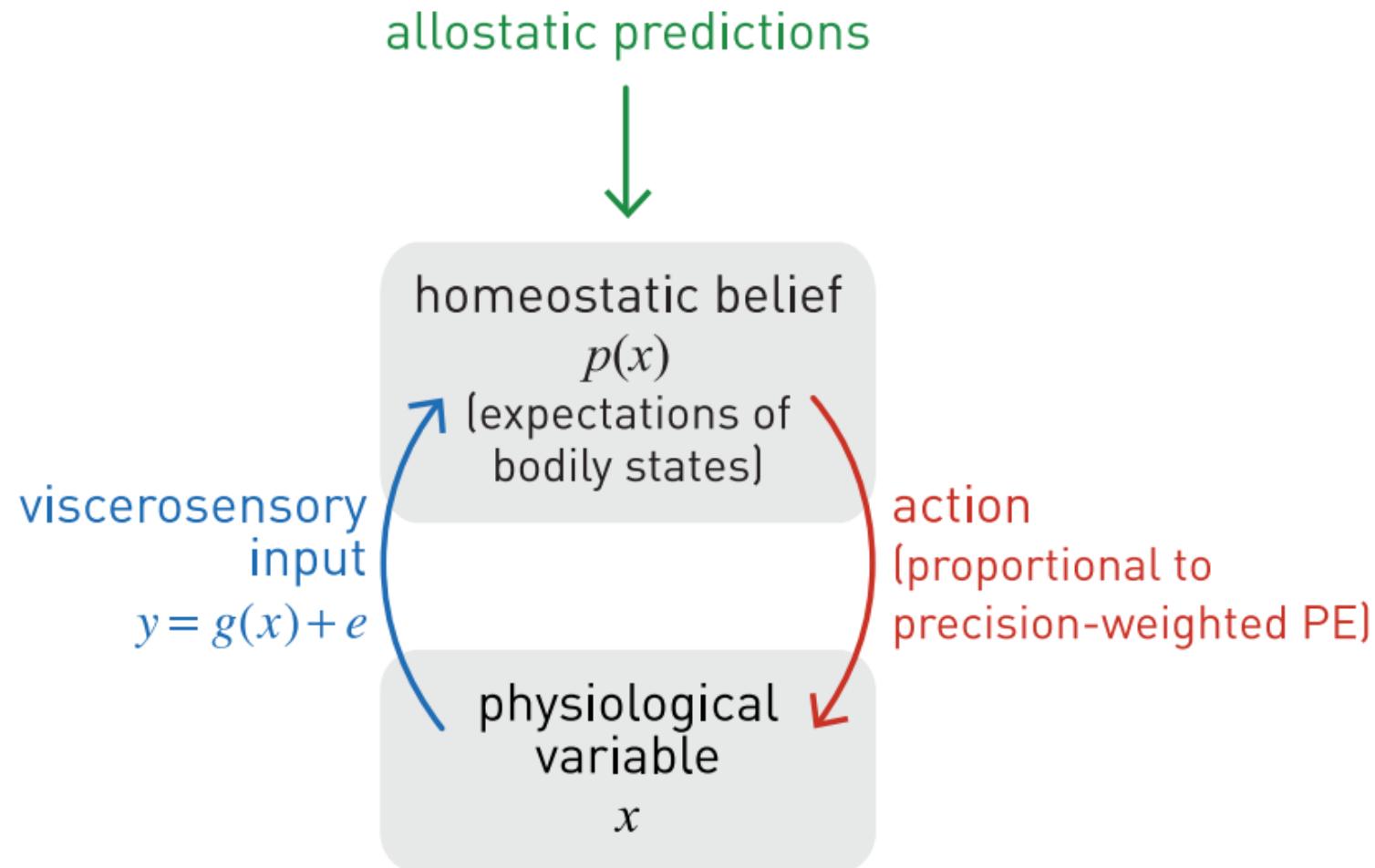
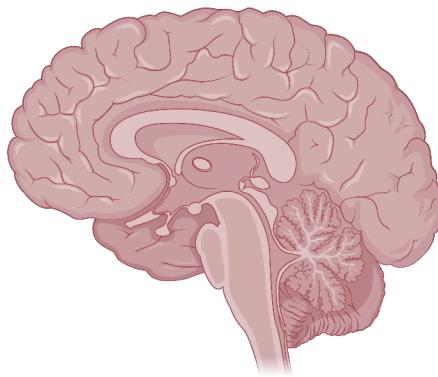


Figure from Stephan et al., *Frontiers in Human Neuroscience*, 2016

Self-efficacy



$$\frac{p(x|y, m)}{p(y|x, m)p(x|m)}$$



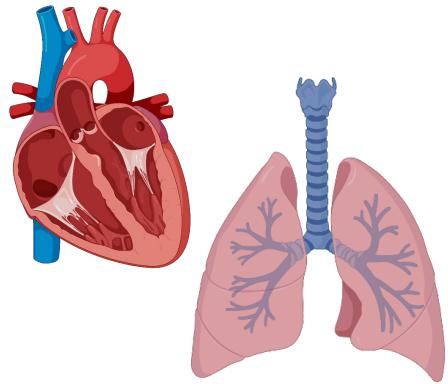
$$\frac{p(x|y, m)}{p(y|x, m)p(x|m)}$$



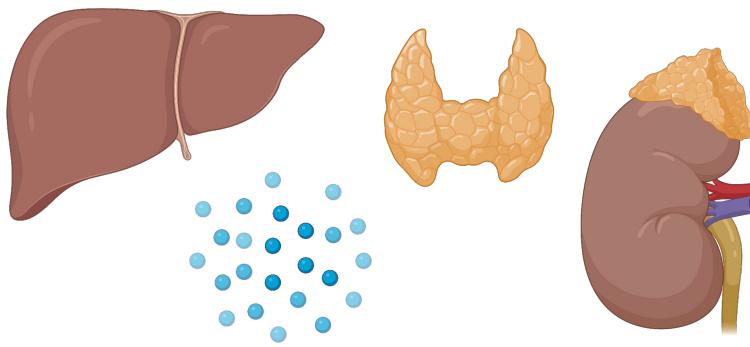
Interoceptive surprise

Exteroceptive surprise

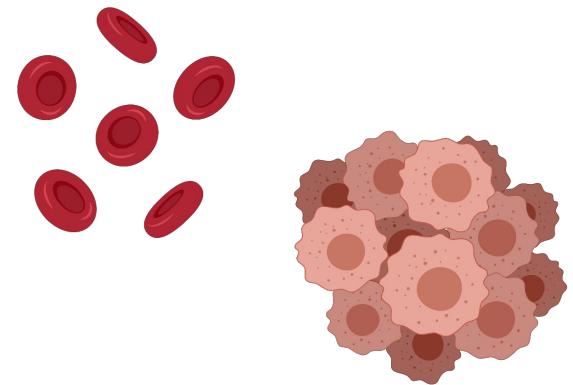
Based on figure from Stephan et al., *Frontiers in Human Neuroscience*, 2016



Cardiopulmonary



Endocrinologic/metabolic



Hematologic/neoplastic

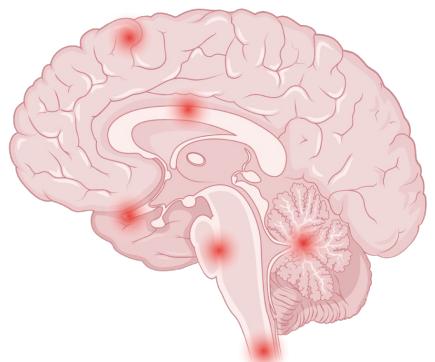


Substance use

Fatigue



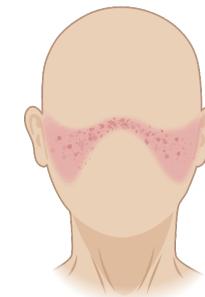
Medication



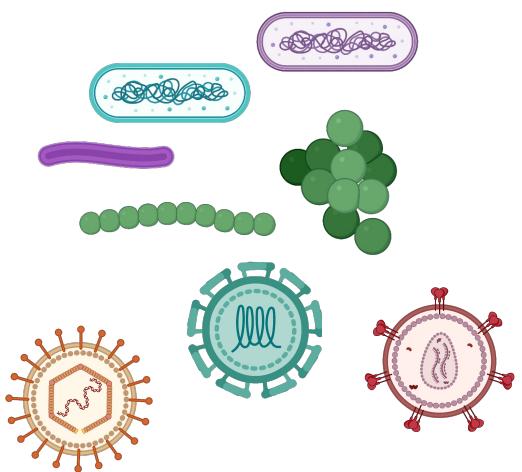
Neurologic



Psychiatric



Rheumatologic



Infectious

Dyshomeostasis

Stephan et al., *Frontiers in Human Neuroscience*, 2016

Dyshomeostasis

*[State of] chronically enhanced surprise about bodily signals, or,
equivalently, low evidence for the brain's model of bodily states*

Desiderata for a computational theory of fatigue

- Needs to account for:
 - Relation to dyshomeostasis in general
 - Role of brain-body interactions
 - Possibility that dyshomeostasis could be merely perceived
(\neq fatigue less real!)
- Needs to help with differential diagnosis

Metacognition

Petzschnner et al., *Biological Psychiatry*, 2017

Metacognition

Self-monitoring of one's level of mastery in acting on the world... and can be seen as a high-level form of inference about one's capacity for control

• • •

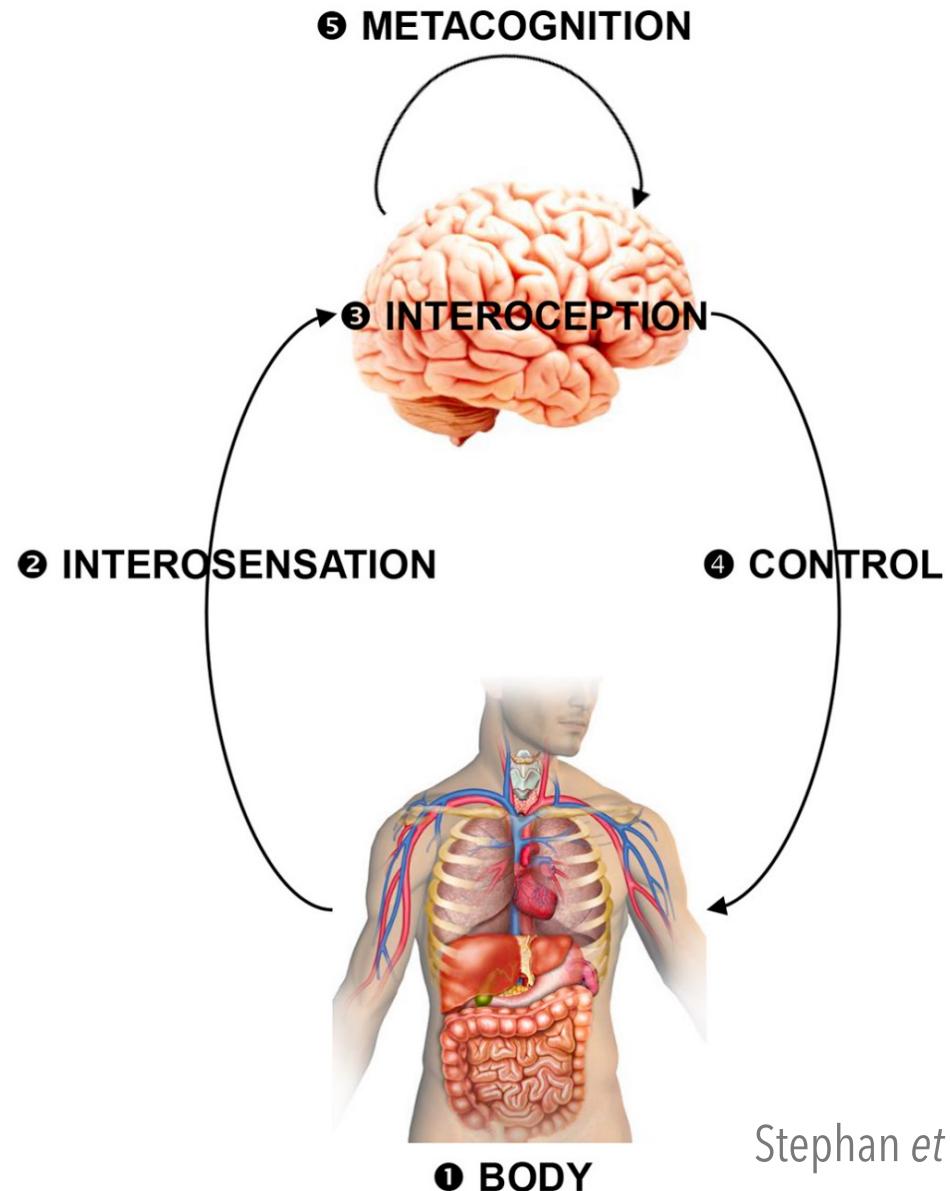
Key concepts

- Homeostasis
- Allostasis
- Generative models
- What is a good model?

Allostatic Self-efficacy: A Metacognitive Theory of Dyshomeostasis-Induced Fatigue and Depression

Klaas E. Stephan^{1, 2, 3}, Zina M. Manjaly^{1, 4}, Christoph D. Mathys², Lilian A. E. Weber¹, Saeed Paliwal¹, Tim Gard^{1, 5}, Marc Tittgemeyer³, Stephen M. Fleming², Helene Haker¹, Anil K. Seth⁶ and Frederike H. Petzschner¹*

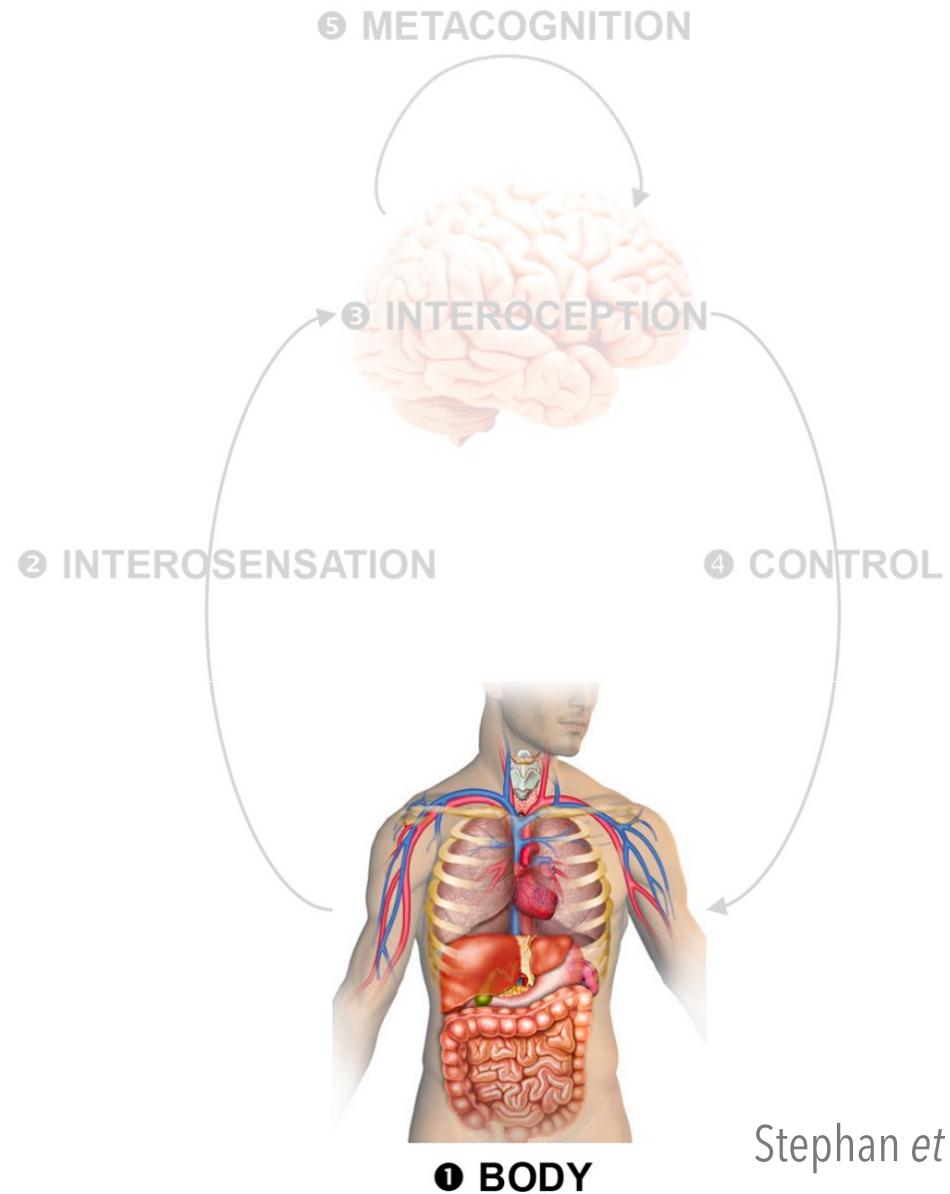
¹ Translational Neuromodeling Unit, Institute for Biomedical Engineering, University of Zurich and ETH Zurich, Zurich, Switzerland, ² Wellcome Trust Centre for Neuroimaging, University College London, London, UK, ³ Max Planck Institute for Metabolism Research, Cologne, Germany, ⁴ Department of Neurology, Schulthess Clinic, Zurich, Switzerland, ⁵ Center for Complementary and Integrative Medicine, University Hospital Zurich, Zurich, Switzerland, ⁶ Sackler Centre for Consciousness Science, School of Engineering and Informatics, University of Sussex, Brighton, UK



Stephan et al., *Frontiers in Human Neuroscience*, 2016

Petzschnner et al., *Biological Psychiatry*, 2017

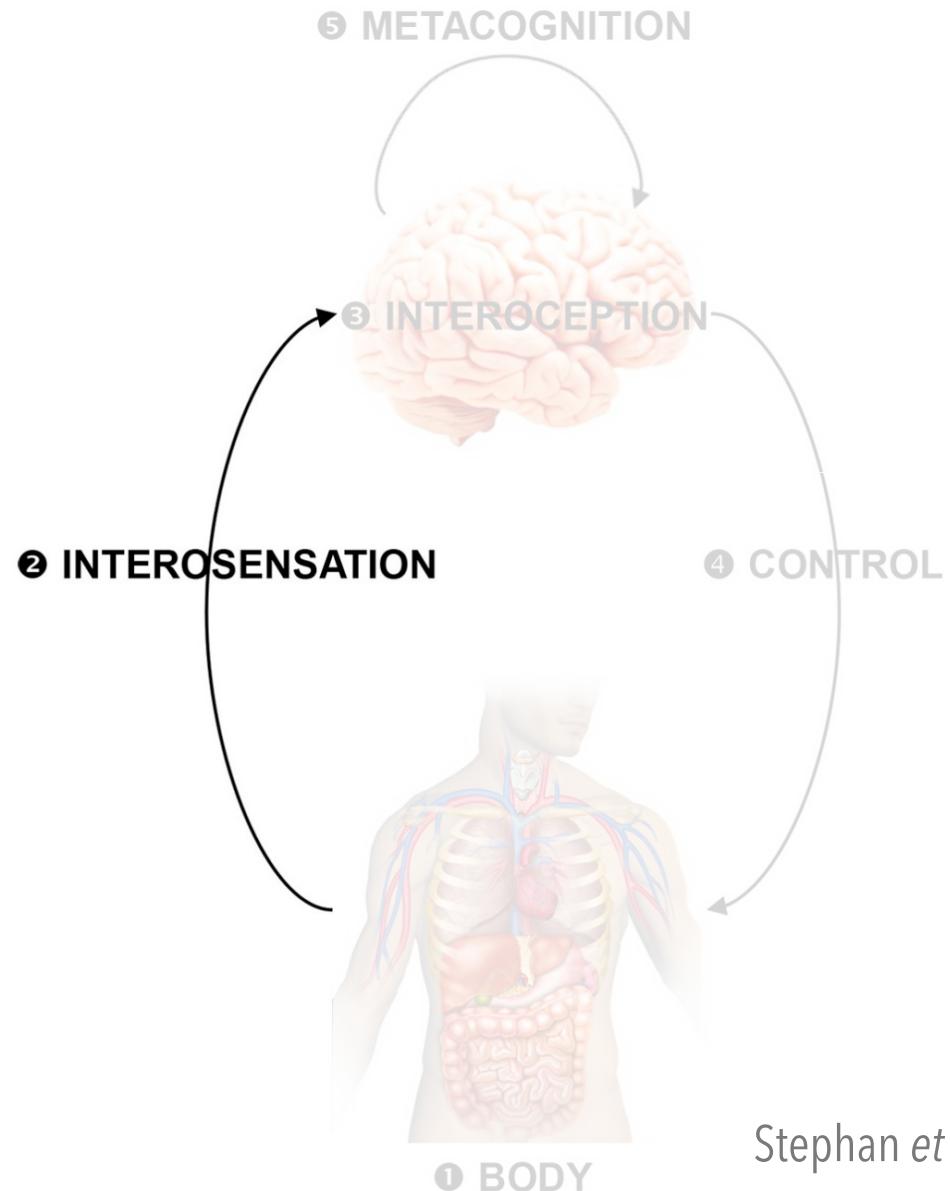
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



Stephan et al., *Frontiers in Human Neuroscience*, 2016

Petzschnner et al., *Biological Psychiatry*, 2017

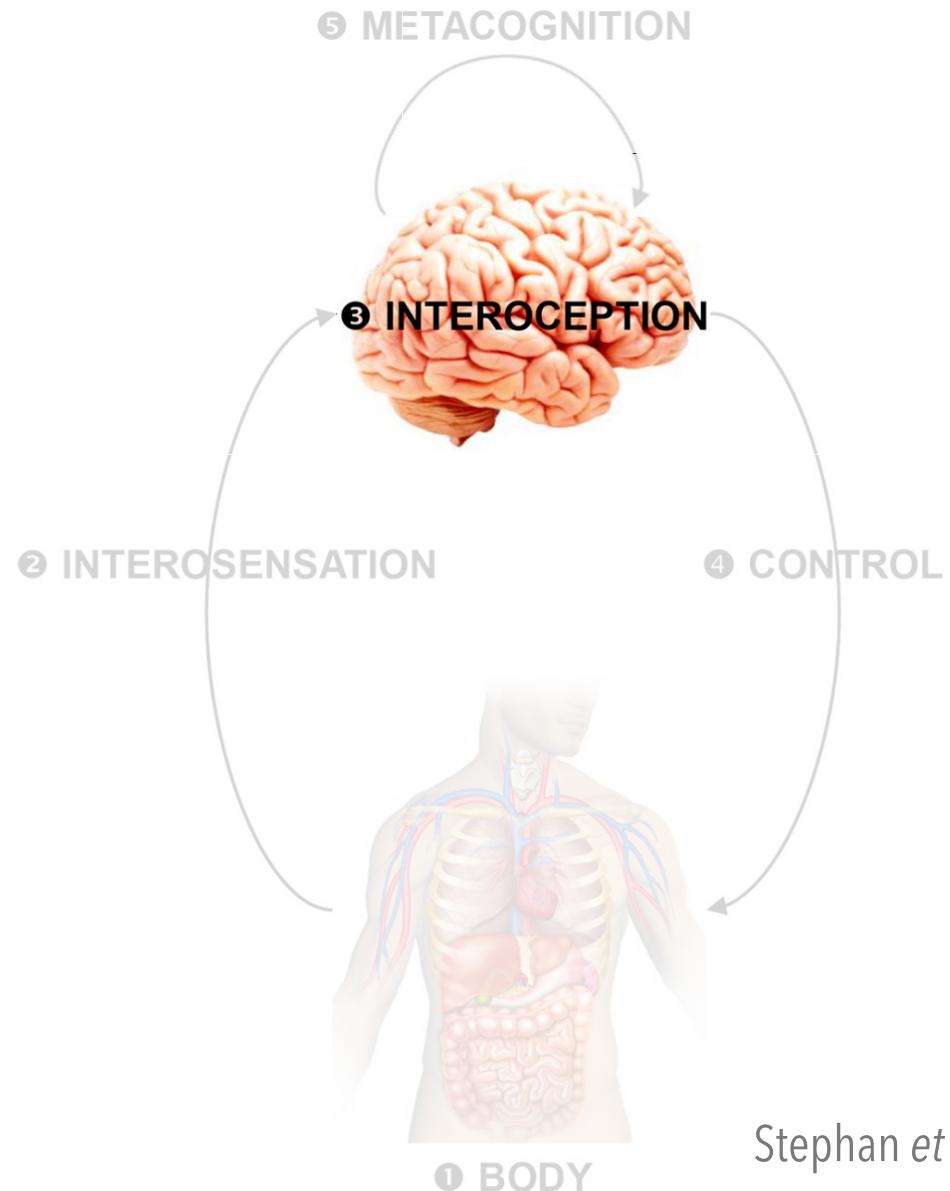
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



Stephan et al., *Frontiers in Human Neuroscience*, 2016

Petzschnner et al., *Biological Psychiatry*, 2017

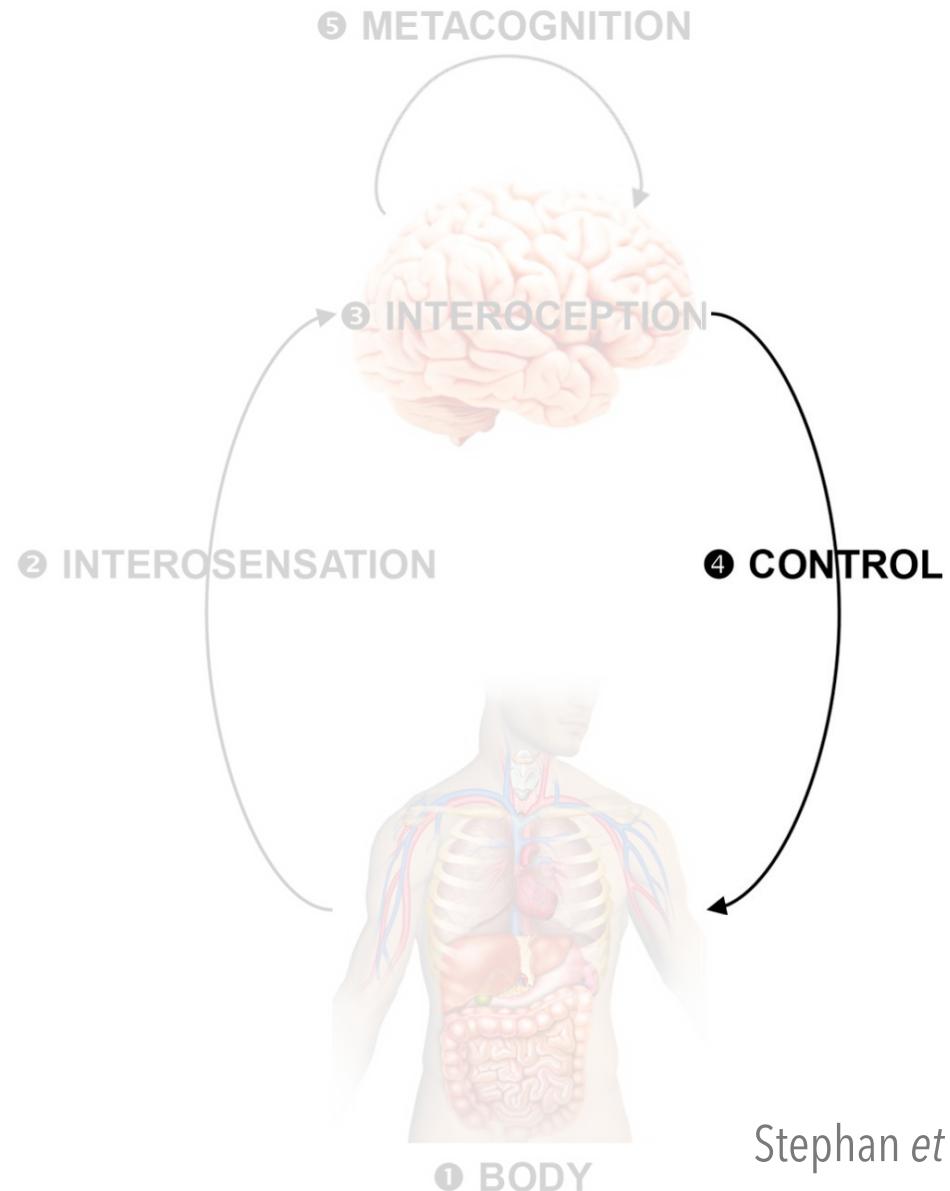
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



Stephan et al., *Frontiers in Human Neuroscience*, 2016

Petzschnner et al., *Biological Psychiatry*, 2017

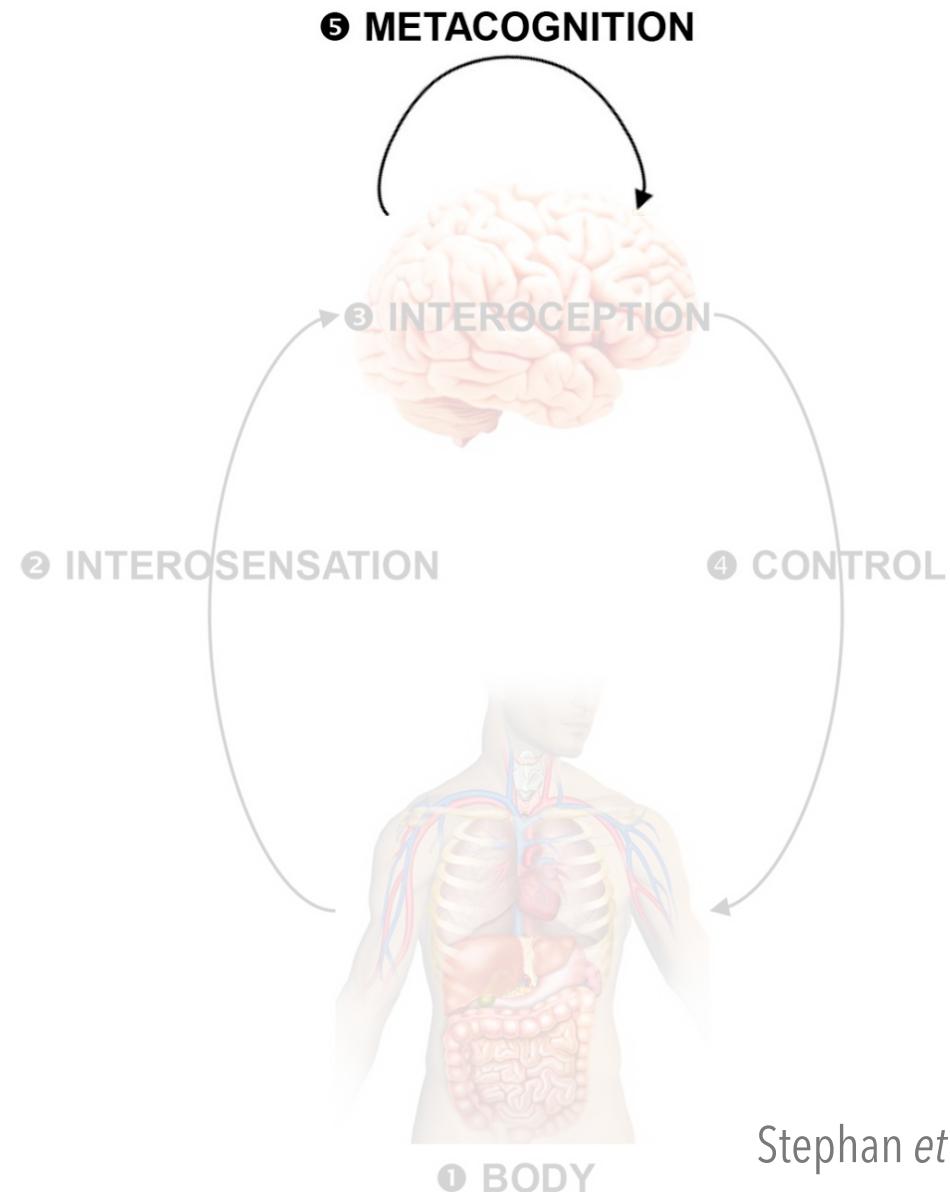
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



Stephan et al., *Frontiers in Human Neuroscience*, 2016

Petzschnner et al., *Biological Psychiatry*, 2017

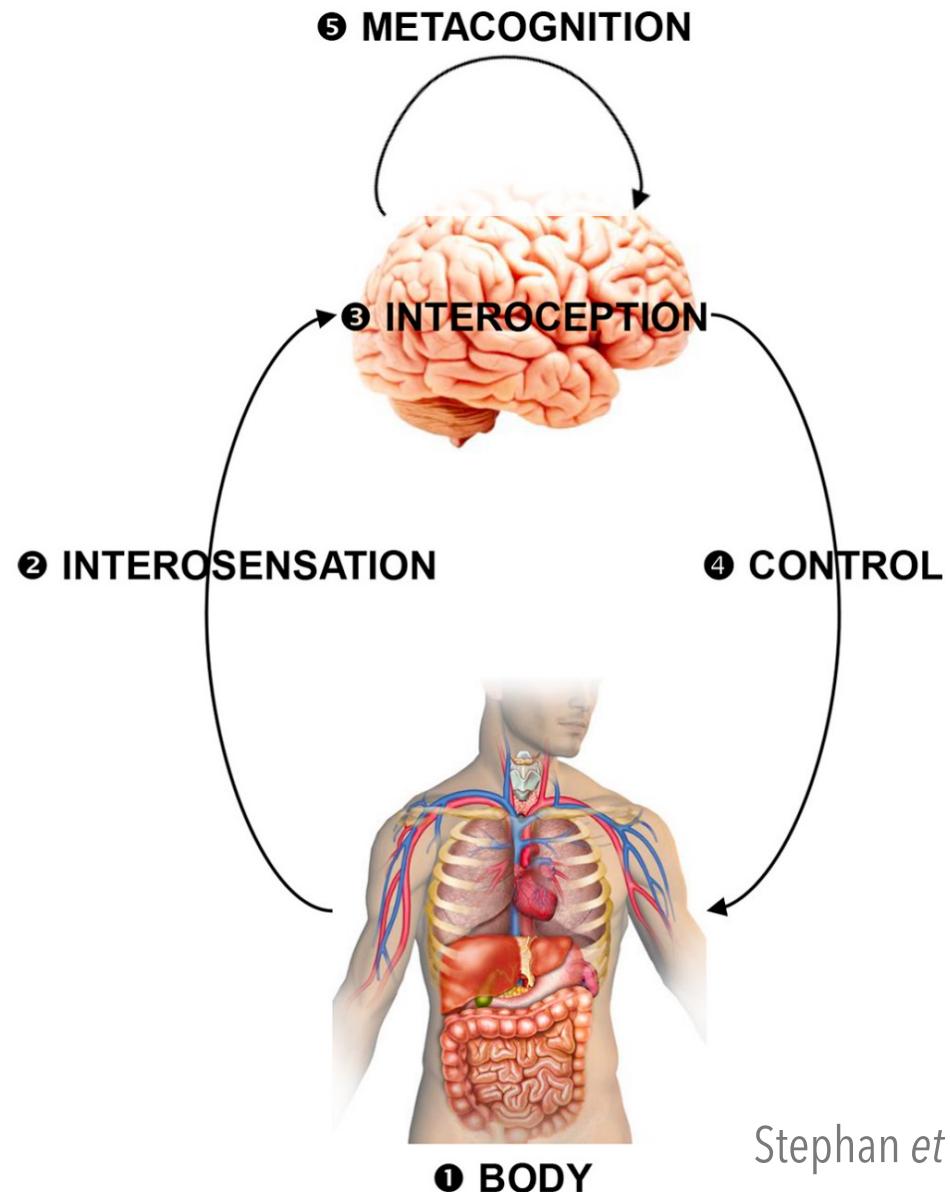
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



Stephan et al., *Frontiers in Human Neuroscience*, 2016

Petzschnner et al., *Biological Psychiatry*, 2017

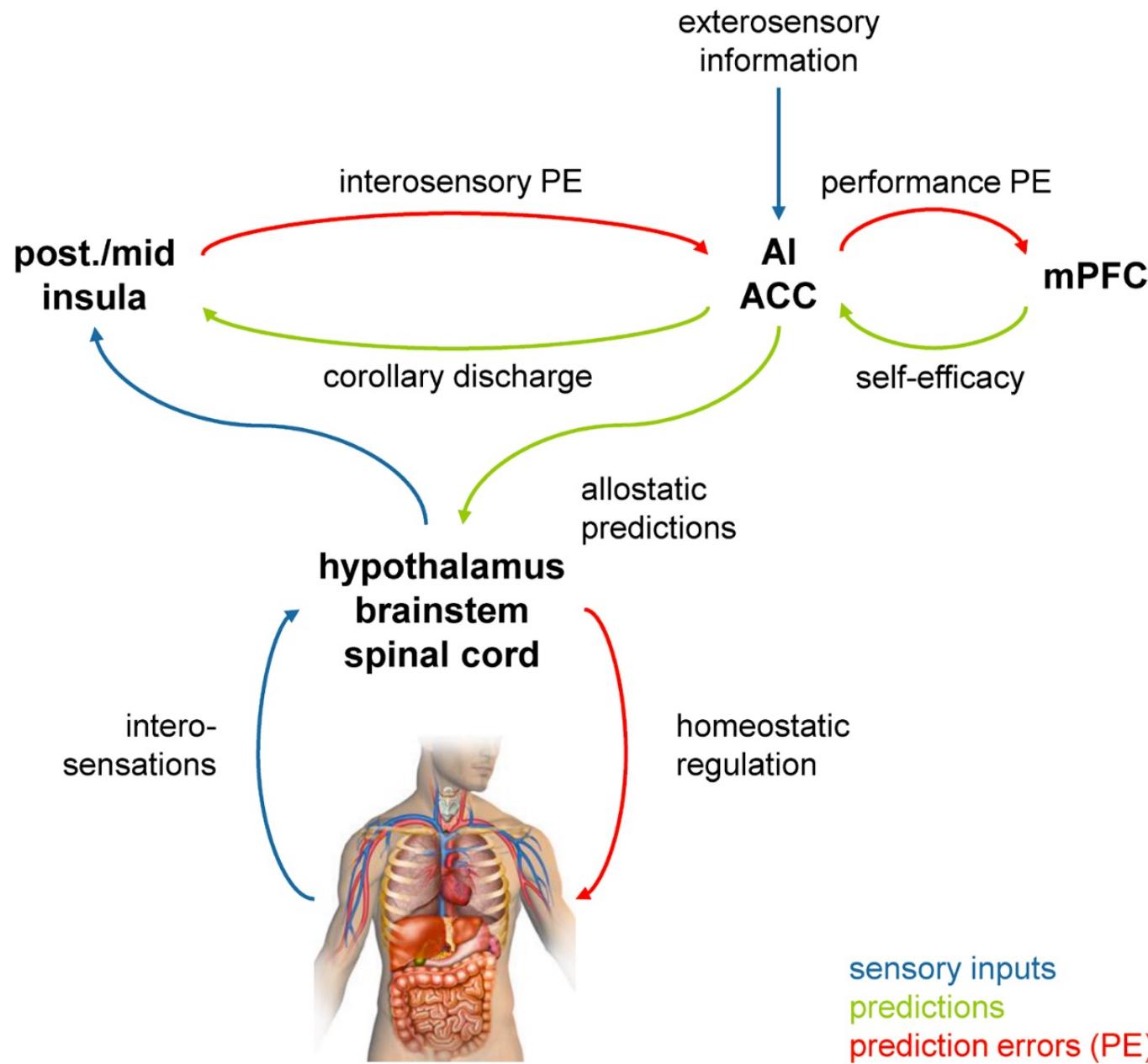
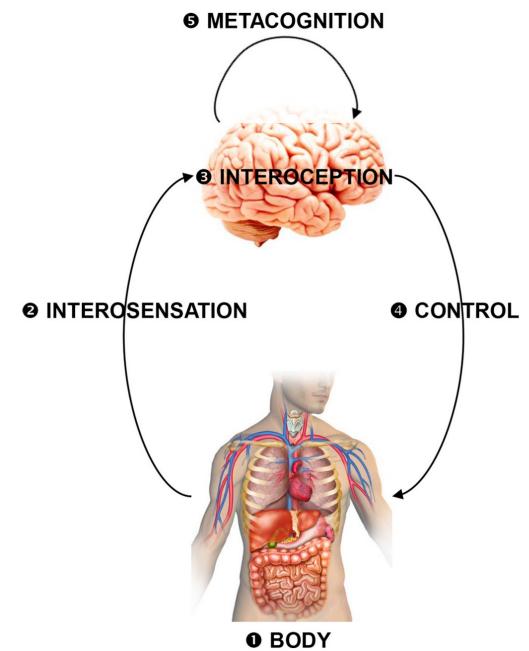
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



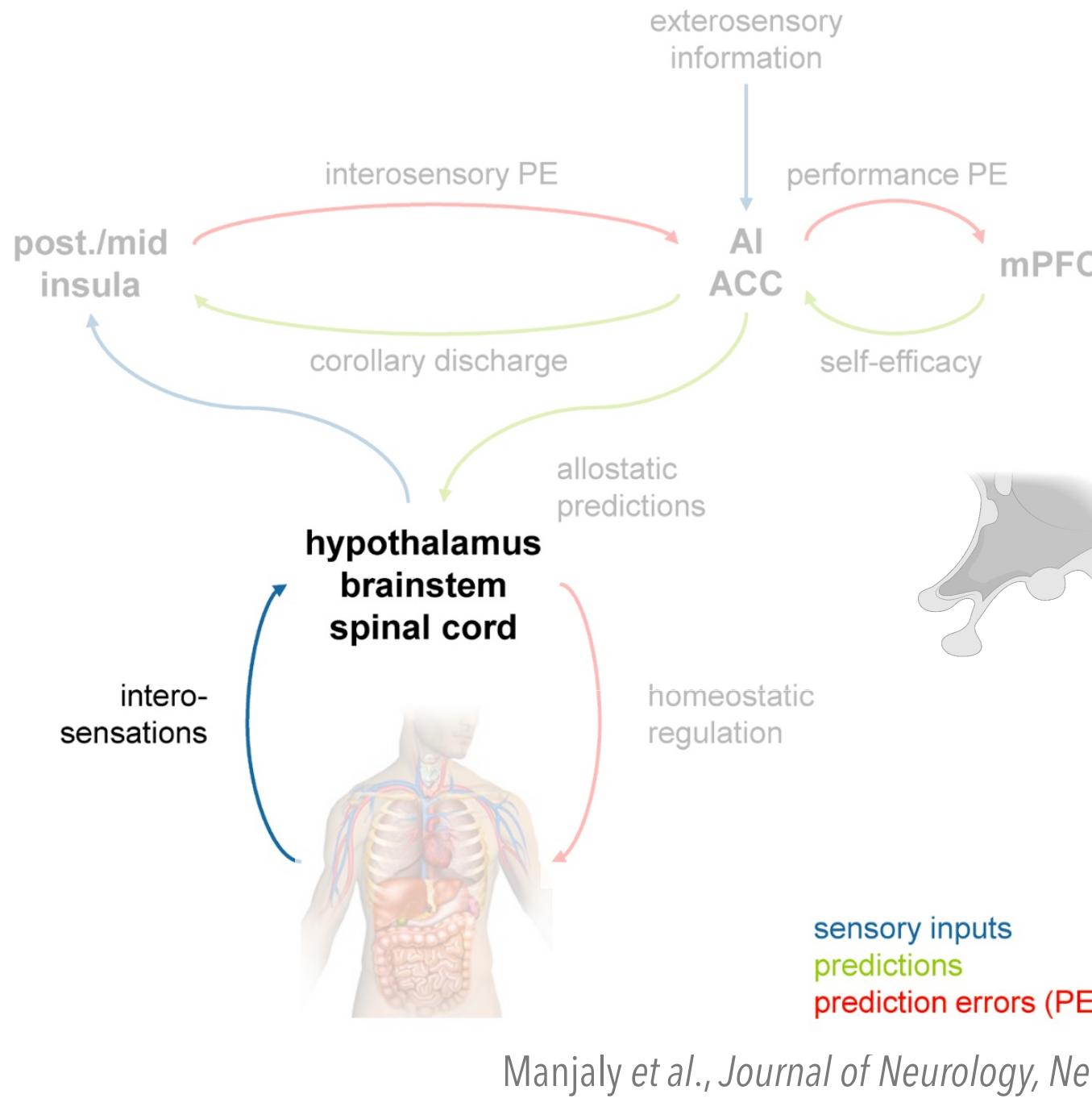
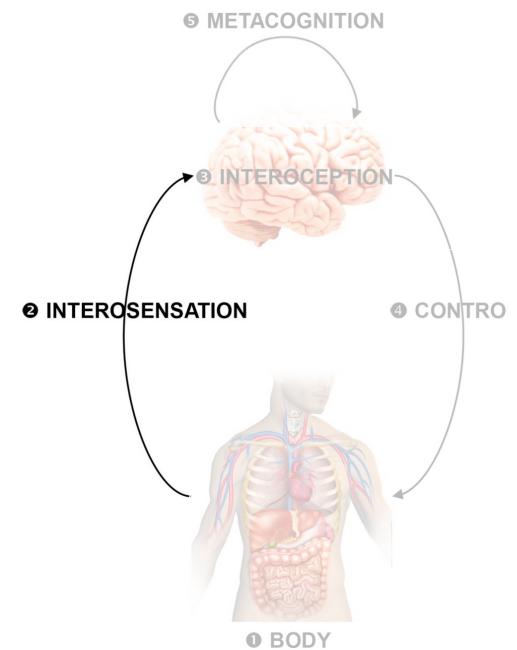
Stephan et al., *Frontiers in Human Neuroscience*, 2016

Petzschnner et al., *Biological Psychiatry*, 2017

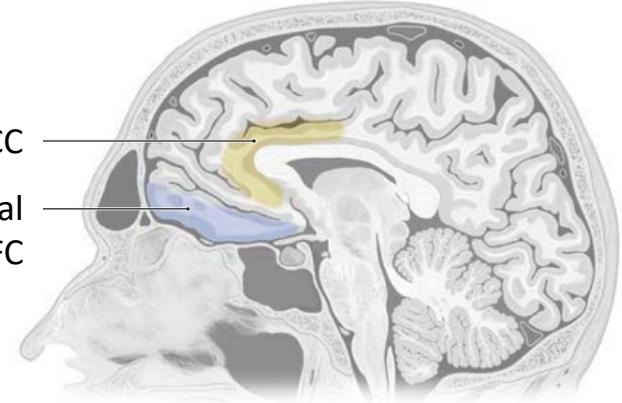
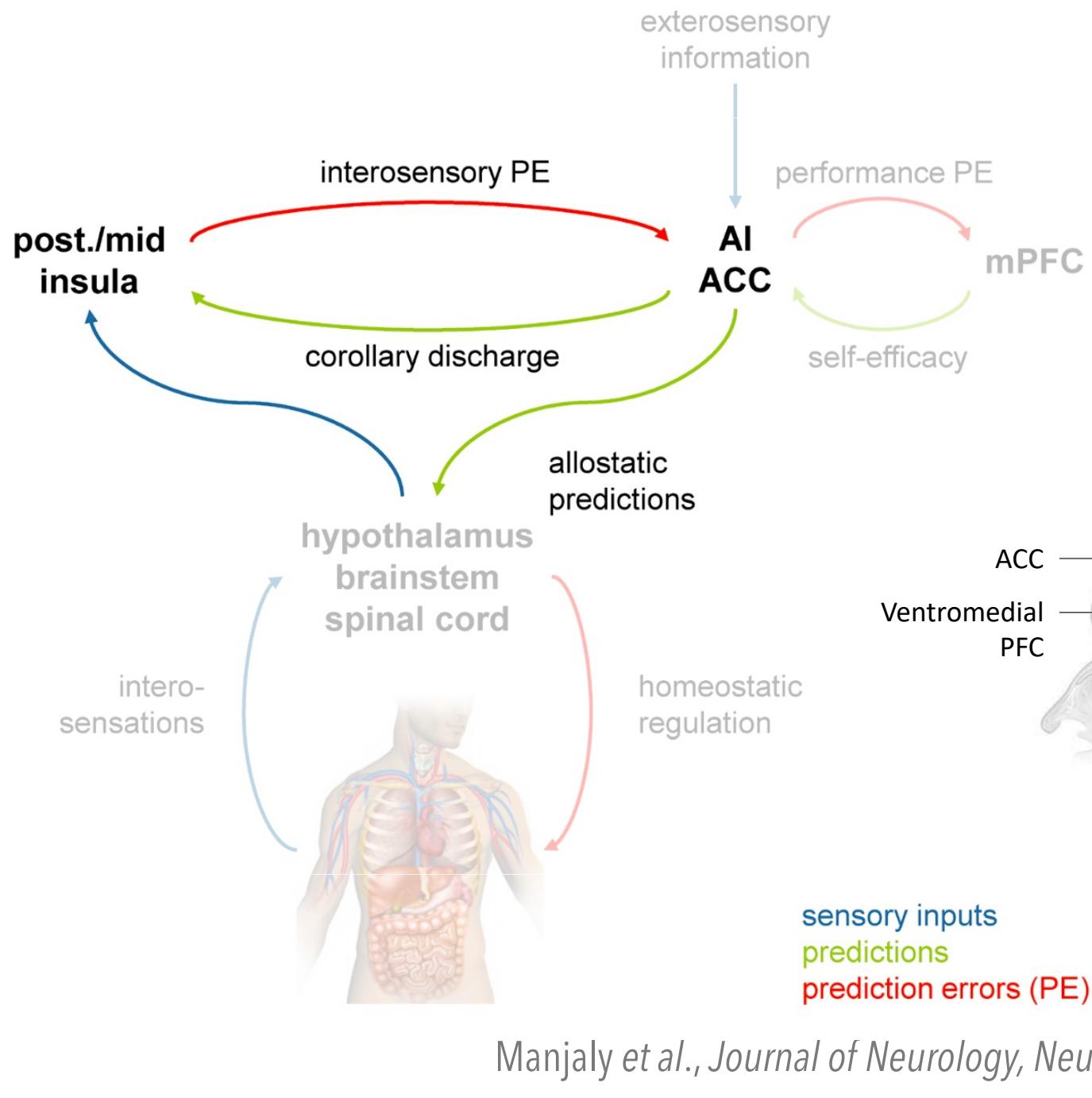
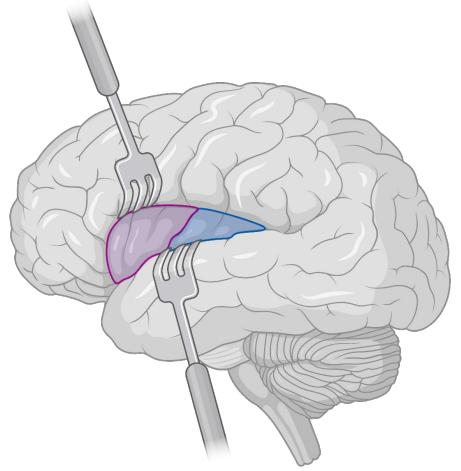
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



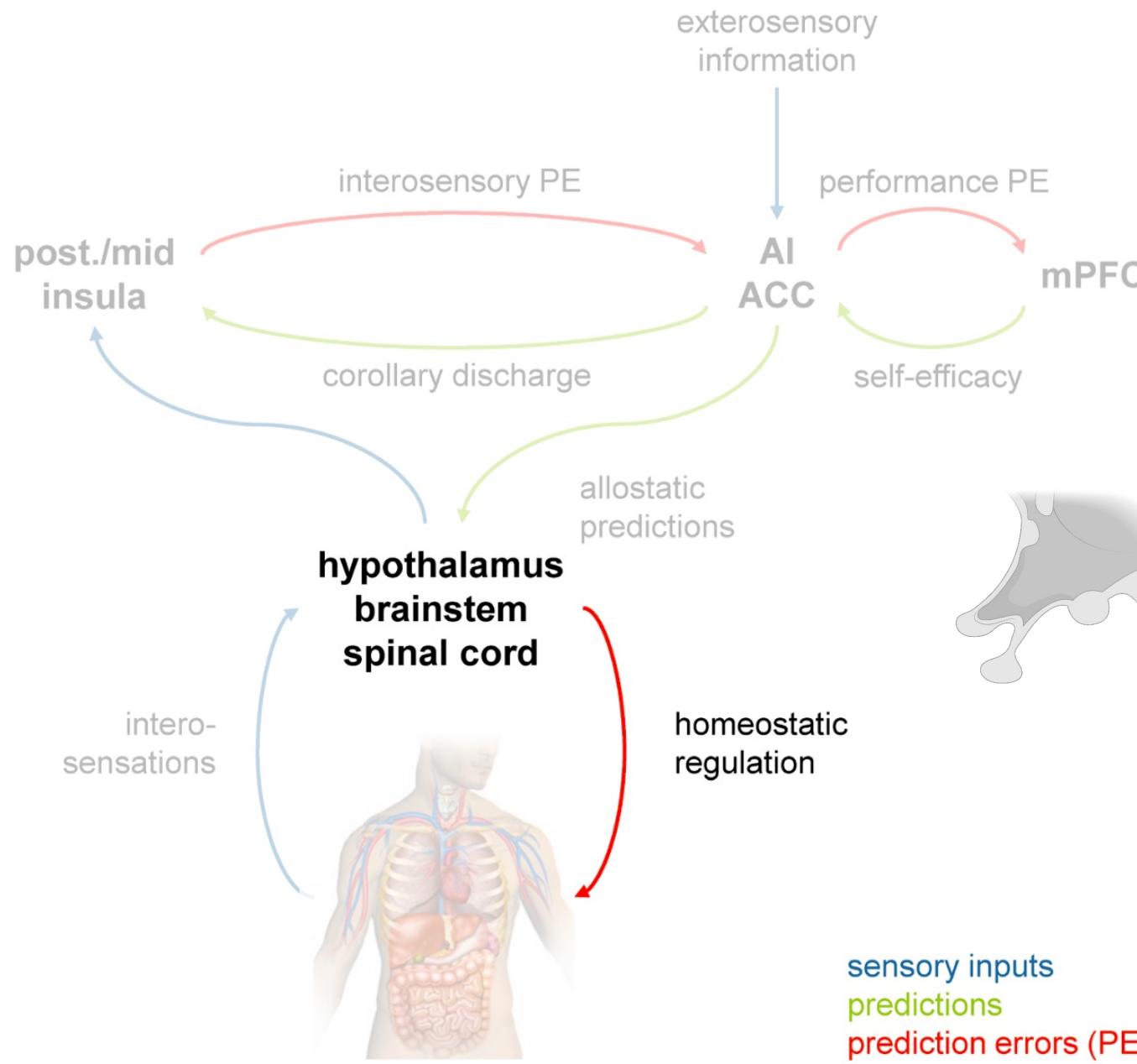
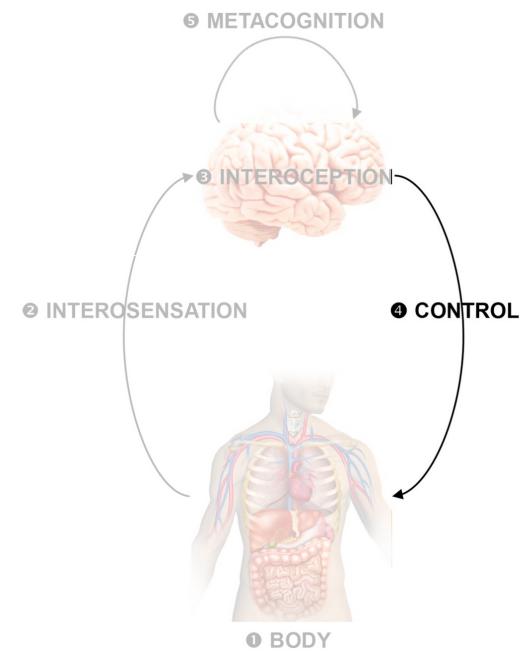
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



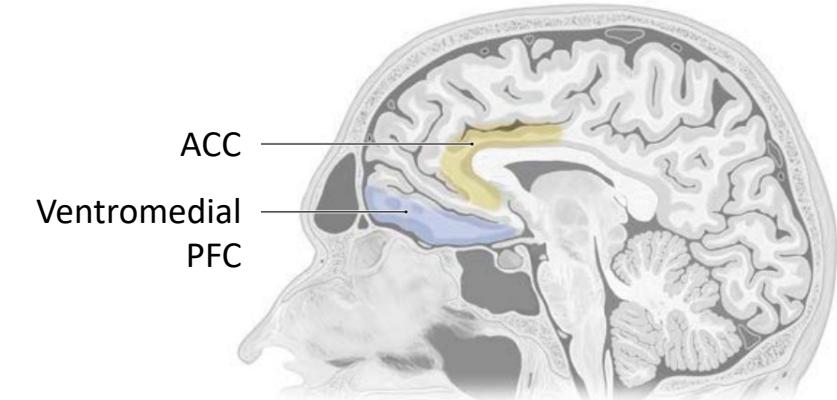
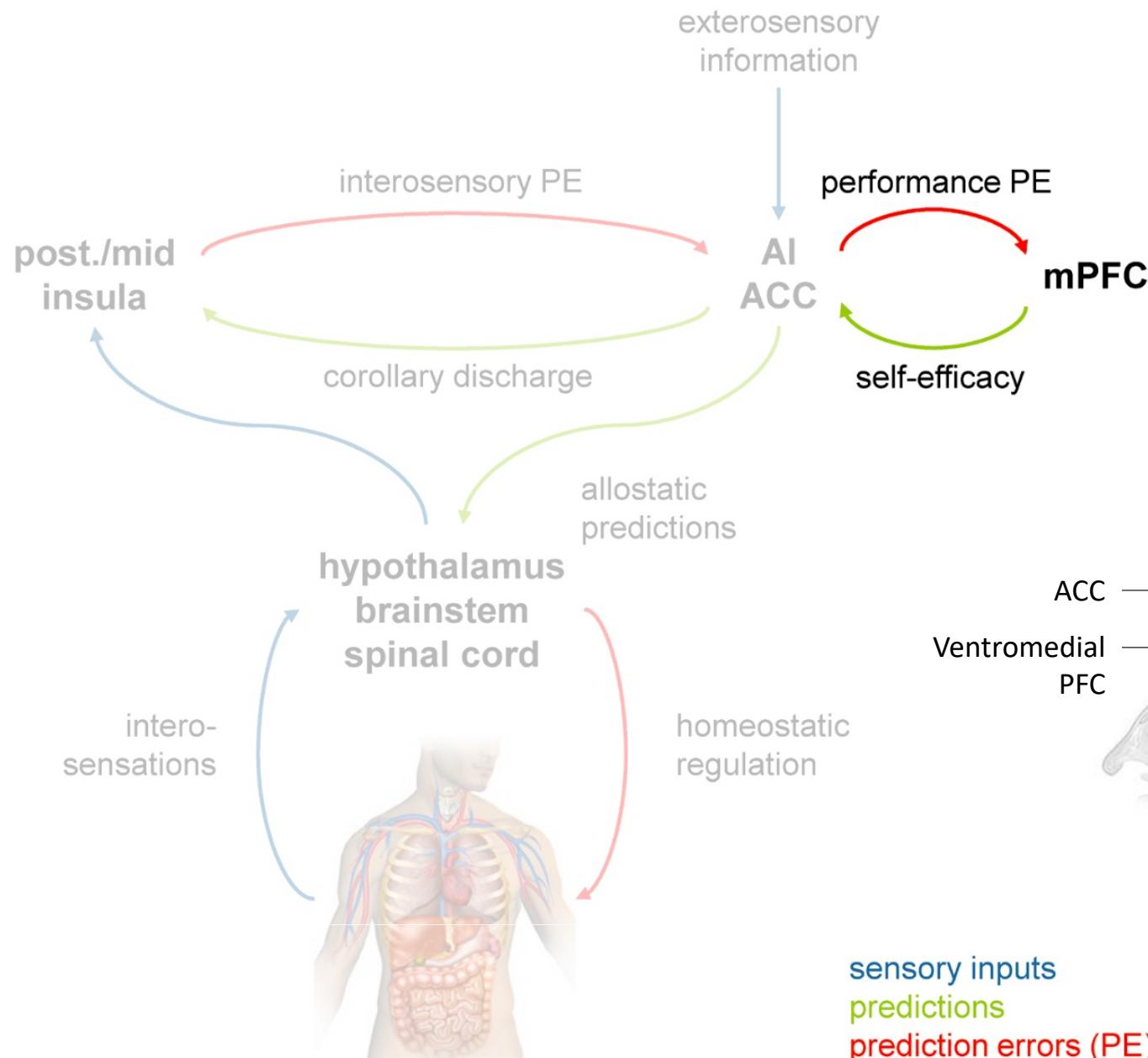
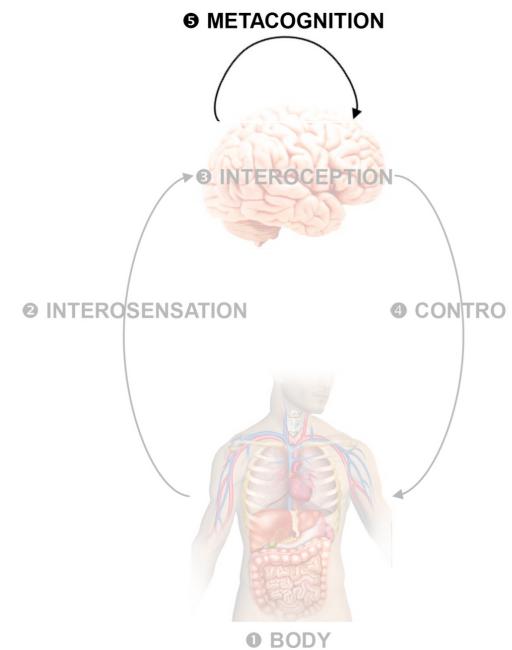
Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019



Manjaly et al., *Journal of Neurology, Neurosurgery and Psychiatry*, 2019

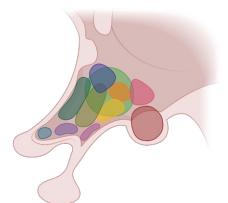
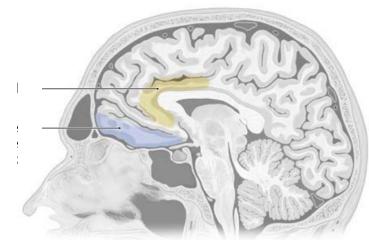
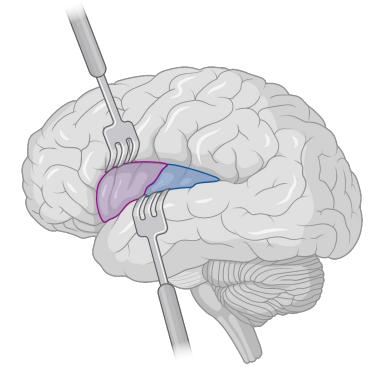


Manjaly et al., Journal of Neurology, Neurosurgery and Psychiatry, 2019

Empirical evidence?

Anatomical areas from the circuit model

- Anterior and posterior insula:
 - Inflammation-induced microstructural changes are correlated with fatigue levels (Harrison *et al.*, 2015)
 - Among the most frequently affected cortical regions in MS (Haider *et al.*, 2016)
- Cingulate gyrus
 - Among the most frequently affected cortical regions in MS (Haider *et al.*, 2016)
- Hypothalamus
 - Biochemical/structural (Kantorová *et al.*, 2017) and structural connectivity changes (Hanken *et al.*, 2015) associated with fatigue



Interoception (Gonzalez Campo *et al.*, 2020)

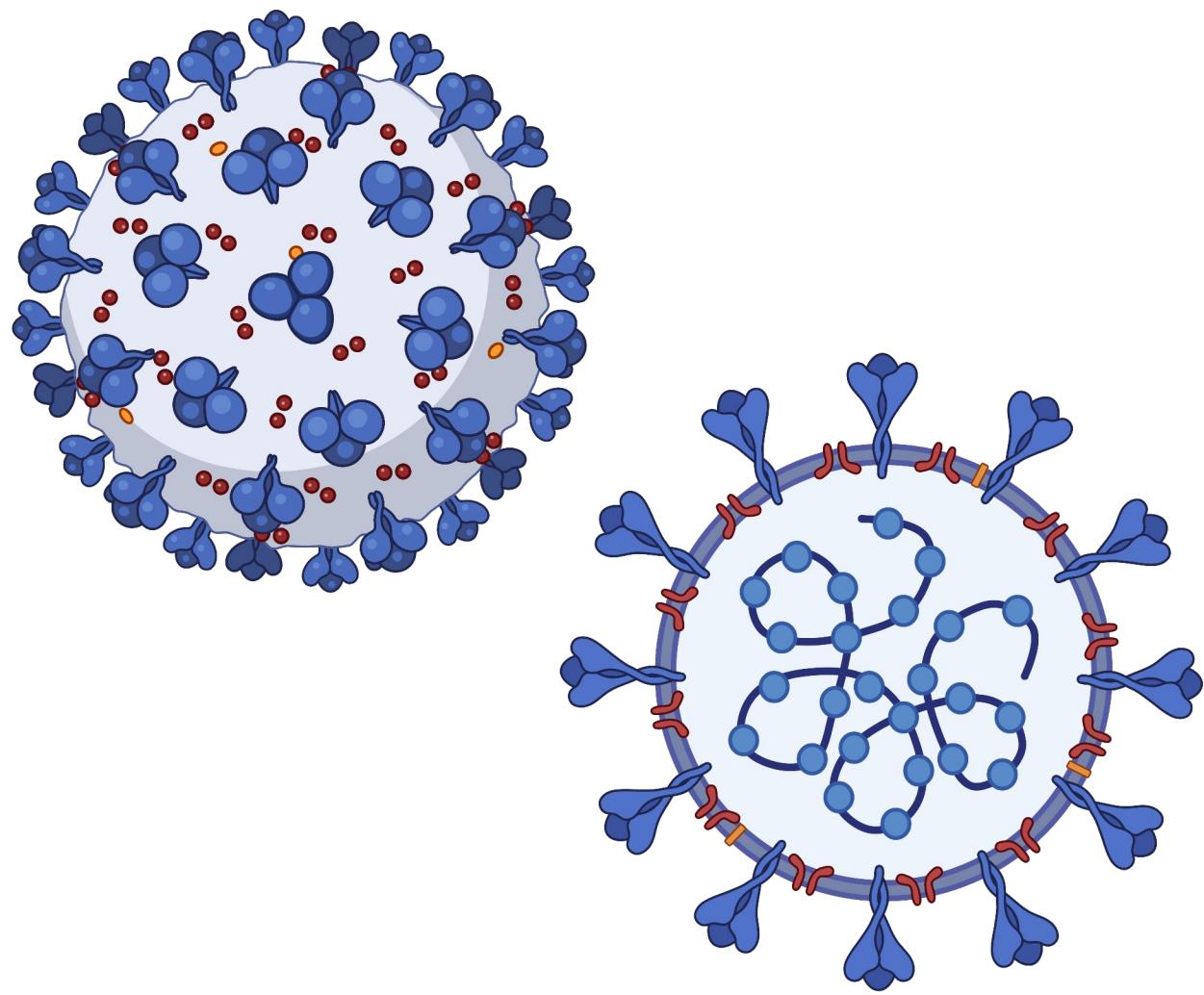
- Measures:
 - Heartbeat detection task
 - Structural and functional MRI
- Fatigued MS patients presented:
 - ↓ interoceptive accuracy
 - ↓ gray matter volume
 - ↑ functional connectivity in insula and anterior cingulate cortex
- Each of these alterations was positively associated with fatigue

Metacognition

- Various measures of autonomic dysfunction correlate strongly with individual fatigue levels (Flachenecker *et al.*, 2003; Newton *et al.*, 2009; Cortez *et al.*, 2015)
- ... but no study has examined metacognition about interoception or homeostasis/allotasis thus far!
 - Maher-Edwards *et al.*, 2011: metacognitive factors predict individual fatigue levels.
 - However: metacognitive assessment did not specifically consider interoception
 - Delgado-Pastor *et al.*, 2015: increasing metacognitive abilities about interoception reduced worry more than increasing metacognition about other cognitive processes
 - However: study did not specifically examine fatigue

Summary

- Fatigue
 - Subjective experience
 - Common nonspecific symptom with a broad range of etiologies
 - Acute, subacute or chronic
- Assessment of subacute and chronic fatigue
 - Comprehensive history
 - Physical examination
 - Basic laboratory studies
 - Updated cancer screening interventions
- Open research question: pathophysiology of fatigue



Thank you for your attention!

Questions?

References

- Gelfand and Douglas, "Fatigue", Harrison's principles of internal medicine, 19th edition, 2018.
- Klimas *et al.*, "Biomarkers of chronic fatigue", *Brain, Behavior and Immunity*, 2012.
- Kung, Nguyen and Das, *Absolute Case-Based Neurology Review*, Springer, 2019.
- Manjaly *et al.*, "Pathophysiological and cognitive mechanisms of fatigue in multiple sclerosis", *Journal of Neurology, Neurosurgery and Psychiatry*, 2019.
- Petzschner *et al.*, "Computational Psychosomatics and Computational Psychiatry: Toward a Joint Framework for Differential Diagnosis", *Biological Psychiatry*, 2017.
- Stephan *et al.*, "Allostatic Self-efficacy: A Metacognitive Theory of Dyshomeostasis-Induced Fatigue and Depression", *Frontiers in Human Neuroscience*, 2016.
- <https://www.uptodate.com/contents/approach-to-the-adult-patient-with-fatigue>
- <https://www.uptodate.com/contents/indications-for-switching-or-stopping-disease-modifying-therapy-for-multiple-sclerosis>
- <https://www.uptodate.com/contents/manifestations-of-multiple-sclerosis-in-adults>
- <https://www.uptodate.com/contents/symptom-management-of-multiple-sclerosis-in-adults>
- ACC and cingulate cortex image: Schünke *et al.*, *Prometheus LernAtlas der Anatomie – Kopf, Hals und Neuroanatomie*, 4th edition, Thieme, 2015 (p. 498)

Acknowledgements

- Many thanks to my TNU colleagues for all their input!
- Figures made with Biorender.com