Proposed Cause of Earliest Stage Amyotrophic Lateral Sclerosis: Plausible Pathophysiology of ALS Involving Glutamate Toxicity and Ionic Dyshomeostasis

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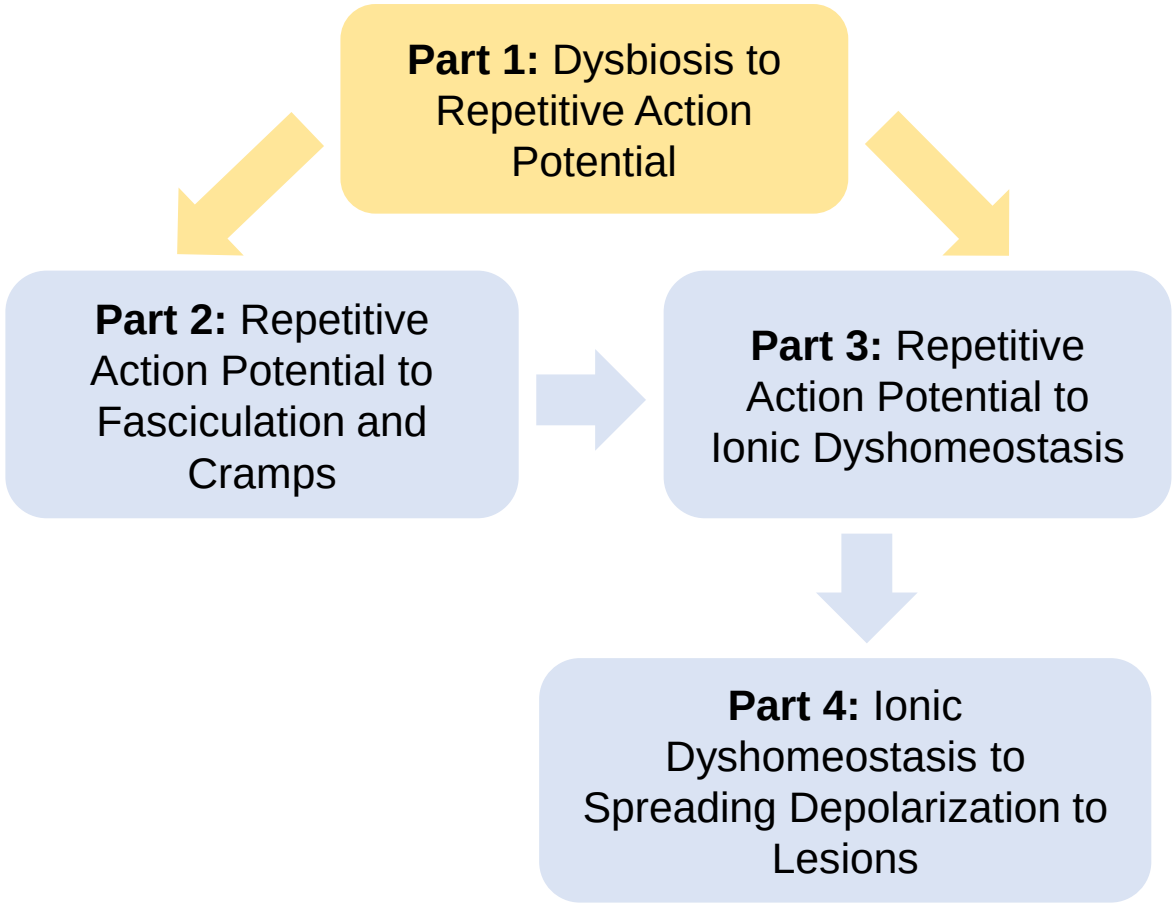
Introduction

- ALS is a disease with unknown pathology
  - Many gene mutations have been linked to ALS, but fALS only accounts for <10% of ALS cases
  - It has been connected to glutamate toxicity for a long time, but entire picture has not been elucidated.
- There is currently no biomarker available for ALS
- Diagnosis delay is on average 16.4 months.
- Understanding the pathology is crucial to the development of biomarkers and treatments
- In this presentation, we propose a pathophysiology of ALS that starts with gut dysbiosis, glutamate toxicity, and ionic dyshomeostasis.
- This would suggest the onset of ALS may not require the loss of motor neurons or gene mutation.

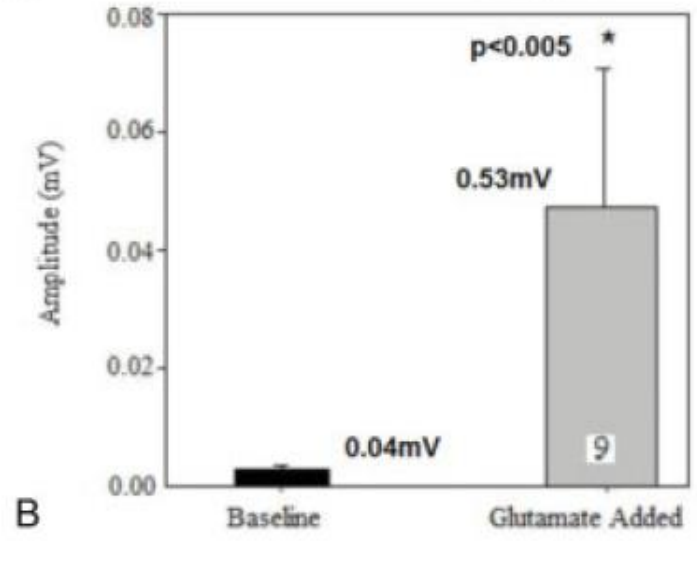


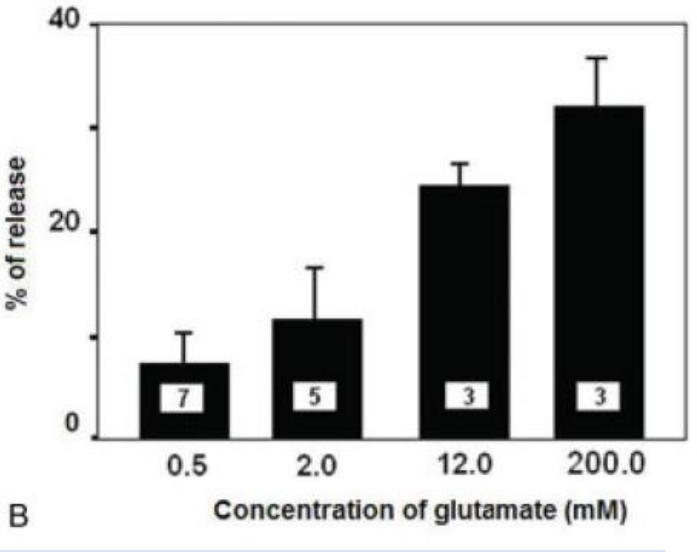
Part 1: Dysbiosis to Repetitive Action Potential

1. Gut dysbiosis & impairment of gut microbiome (observed in ALS)
2. Unmetabolized glutamate enters circulation. Systemically high levels of serum glutamate found in ALS
3. Glutamate can cross the BBB via CVOs
4. Extracellular glutamate rises. (elevated ECS glutamate seen in ALS)
5. Excessive exogenous glutamate over excites global NMDAR (synaptic and axonal).
6. Localized Mg^{2+} depletion that prevent inactivation of overexcited NMDAR.
7. Initiation of **repetitive action potential** which causes further release of glutamate
8. Fasciculation (possible first symptom of ALS) and eventually cramps
9. Ionic dyshomeostasis

Motor cortex, UMN, SC, and LMN

Traditionally, neurotransmitters are thought to communicate between pre- and post-synaptic terminal and produce an “all-or-nothing” action potential. However, it has been observed that axons can also release and receive neurotransmitter-like substances without the involvement of synapses. In an experiment on an *in vitro* sciatic nerve without a cell body or axon terminal, the addition of 100 uM of glutamate produced compound action potential (CAP) for 10 to 75 minutes (Abouelela, 2016).





ALS = amyotrophic lateral sclerosis
BBB = blood brain barrier
CVOs = circumventricular organs
ECS = extracellular space
NBI = New Biotic Inc.
NMDAR = N-methyl-D-aspartate receptor

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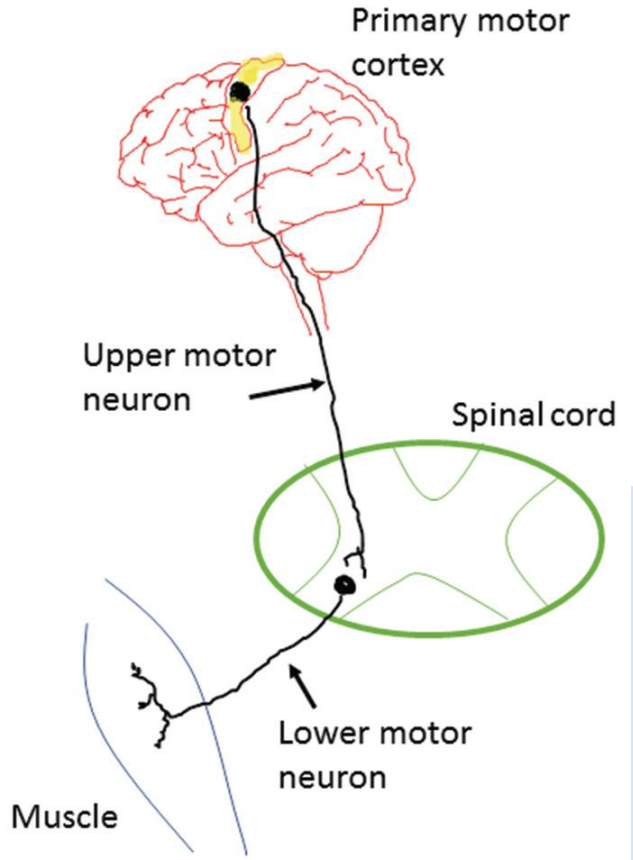

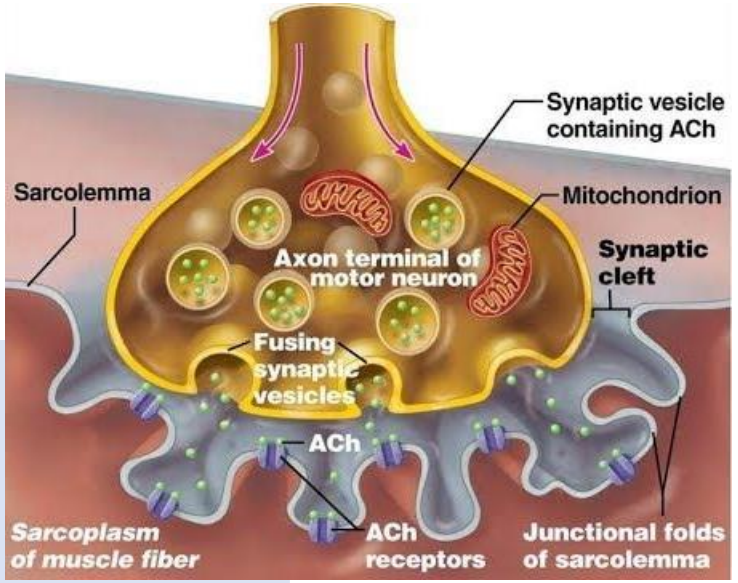

Part 2a: Repetitive Action Potential to Fasciculation

ALS = amyotrophic lateral sclerosis
ACh = acetylcholine
BBB = blood brain barrier
CVOs = circumventricular organs
ECS = extracellular space
LMN = lower motor cortex
NBI = New Biotic Inc.
NMDAR = N-methyl-D-aspartate receptor
UMN = upper motor neuron

5. Repetitive Action Potential

6. Repetitive release of glutamate from UMN & repetitive activation of LMN.

7. Repetitive release of ACh into the NMJ & repetitive repolarization

8. Fasciculation (first symptom of ALS)

Motor cortex, UMN, SC, and LMN

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Part 2b: Fasciculation to cramp

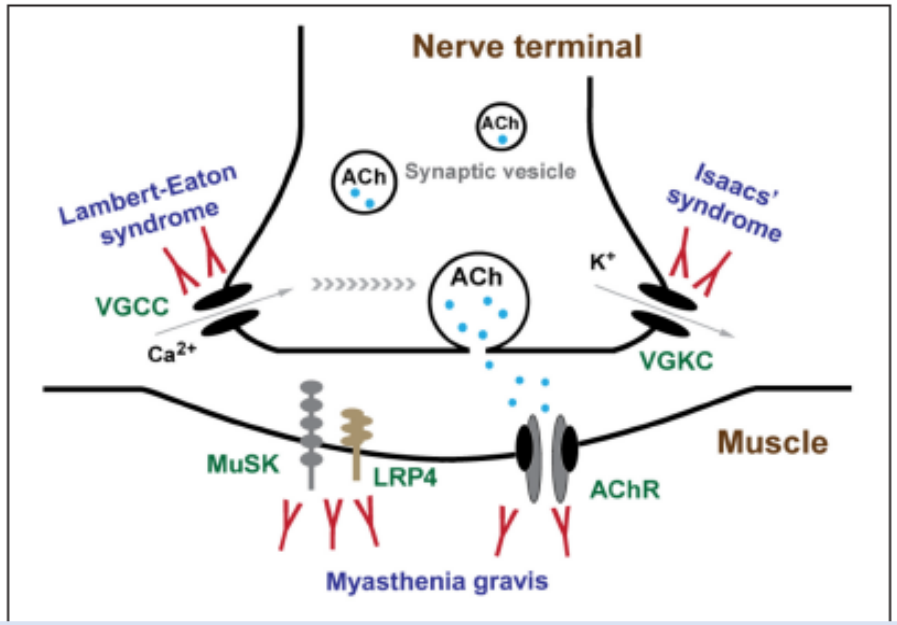
1. Gut dysbiosis & impairment of gut microbiome (observed in ALS)

2. Unmetabolized glutamate enters circulation. Systemically high levels of serum glutamate found in ALS

3. Glutamate can cross the BBB via CVOs

4. Extracellular glutamate rises. (elevated ECS glutamate seen in ALS)

One motor neuron on average innervates 150 muscle fibers. (Cuevas, 2015)



The efflux of K^+ is needed to repolarize the axon terminal and halt the release of ACh (Huang, 2019), but RAP eventually causes low K+ conductance in axon terminal and may delay or prevent the repolarization.

5. Repetitive Action Potential (RAP)

6. Repetitive release of glutamate from UMN and repetitive SC activates LMN.

7. Repetitively release of ACh into the NMJ & repetitive repolarization

8. Fasciculation (first symptom of ALS)

9. As the LMN is repetitively/persistently activated, and K+ is incessantly effluxed, intracellular K^+ may become deficient.

10. The deficiency of intracellular K^+ would delay the repolarization of the axon terminal, thereby resulting in the redundant release of ACh.

11. Cramp and muscle stiffness ensues as muscle contraction cannot stop.

Motor cortex, UMN, SC, and LMN

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Part 2c: Cramp to Ionic Dyshomeostasis

11. Cramp and muscle stiffness ensues as muscle contraction cannot stop.

12. **Hypermetabolism** (seen in ALS). Contraction of muscle requires high utilization of ATP for Na/K ATPase

13. Lipolysis and increased free fatty acid (seen in ALS). Free fatty acid can affect insulin's access to insulin-sensitive cells and may reduce insulin secretion.

14. Impaired glucose tolerance. (Higher plasma glucose and lower insulin in ALS vs control after oral glucose consumption. Another study found higher levels of fasting insulin and lower rates of glucose infusion in ALS)

15. It was found that in people with impaired glucose tolerance, the effect of insulin on brain metabolism is not maximal in fasting state. Therefore, more insulin is needed to achieve a maximal effect on the brain.

16. It was observed that ALS patients have reduced CSF insulin and IGF-I

17. As insulin and IGF-I are both needed to initiate the translocation of GLUT1 to the plasma membrane of astrocytes, reduced insulin and IGF-I results in less GLUT1 translocation

18. Reduced glucose uptake in the brain. (**Brain hypometabolism** in frontal, motor and occipital cortex has been seen in ALS)

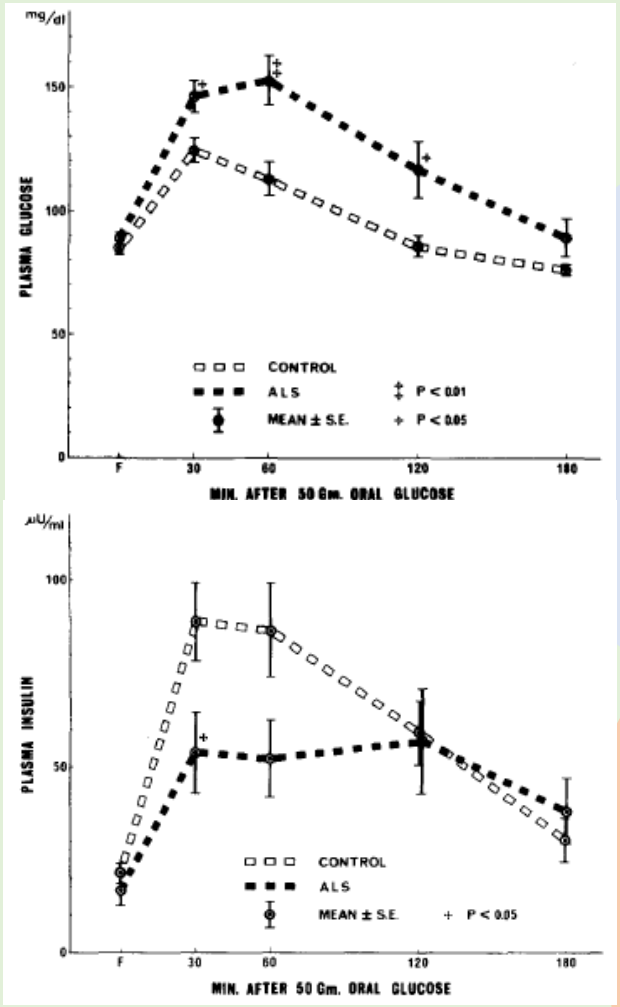
19. Reduced ATP production.

20. Inability of Na/K ATPase on neurons to maintain K^+ homeostasis

21. Chromium deficiency (seen in people with elevated blood glucose, has yet to be investigated in ALS)

Potential therapeutic target: CrHis. Cr^{3+} stabilizes insulin (Ulas, 2015), improves insulin sensitivity (Sahin, 2007) and encourages the translocation of GLUT-1 (Ulas, 2015).

ATP production progressively reduced in SOD1 mice. (Browne, 2006)

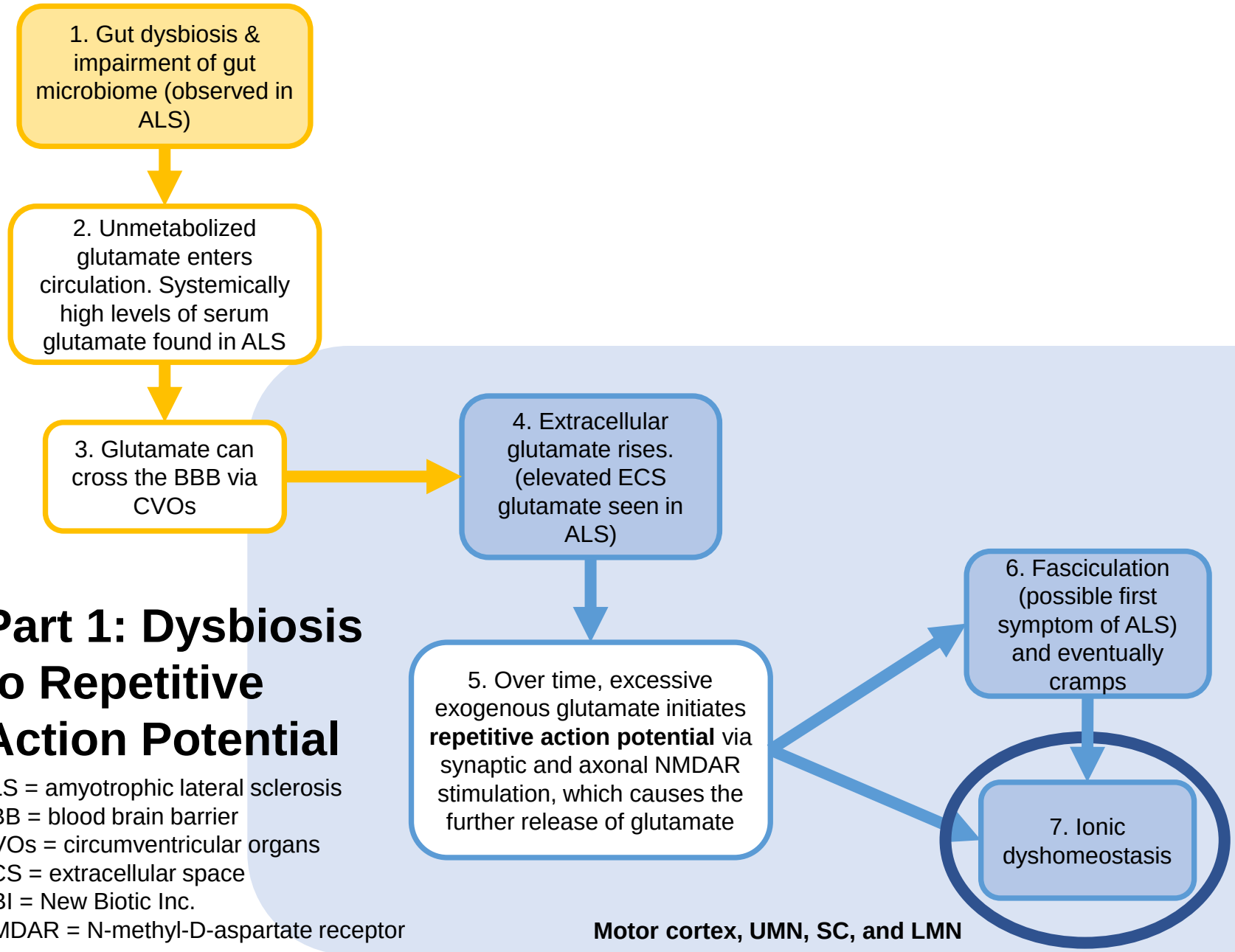


Neuron

Astrocyte

Extracellular space

ALS = amyotrophic lateral sclerosis
CSF = cerebral spinal fluid
CrHis = chromium histidinate
GLUT1 = glucose transporter 1
IGF-I = insulin-like growth factor I



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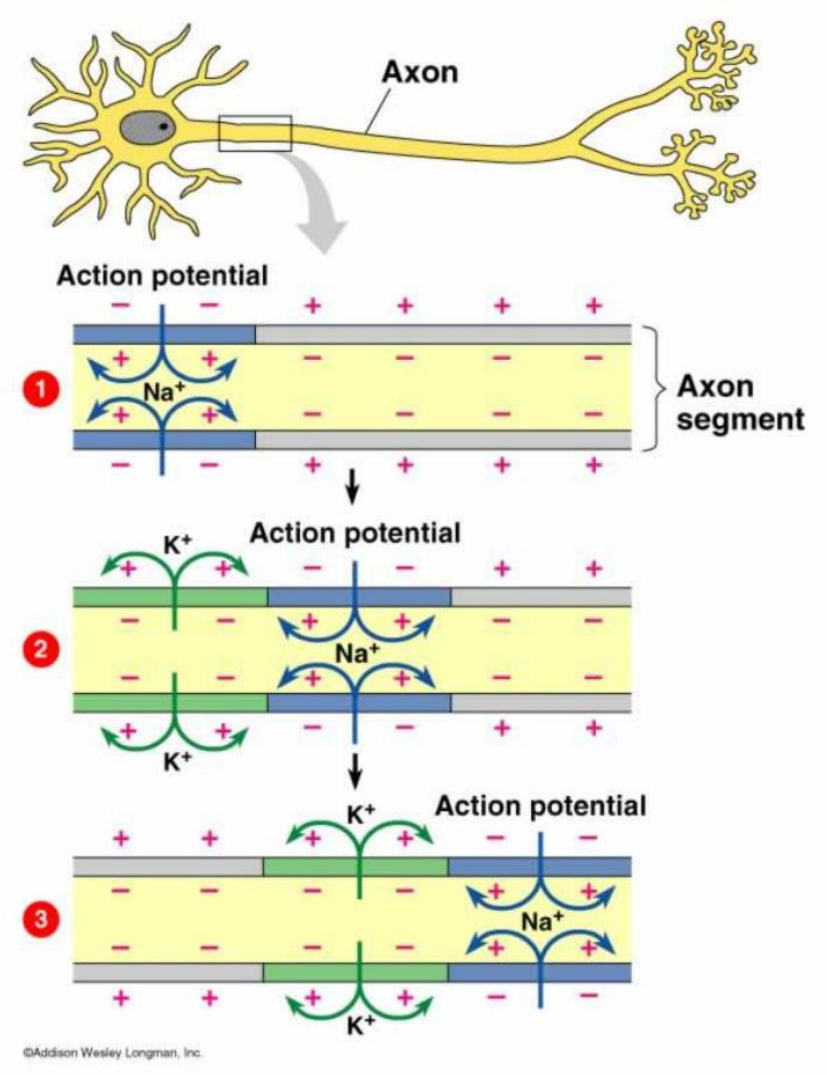
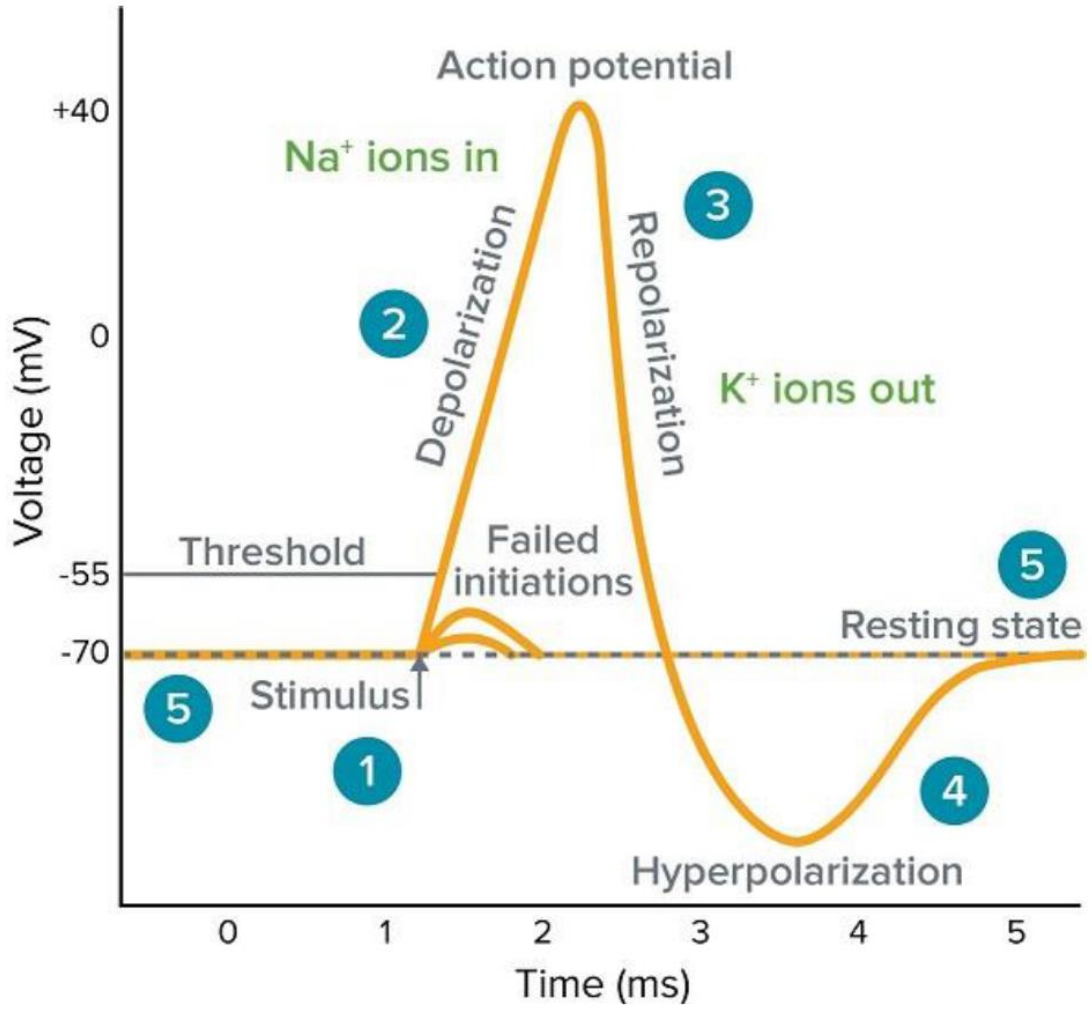
Ionic movement during Action Potential





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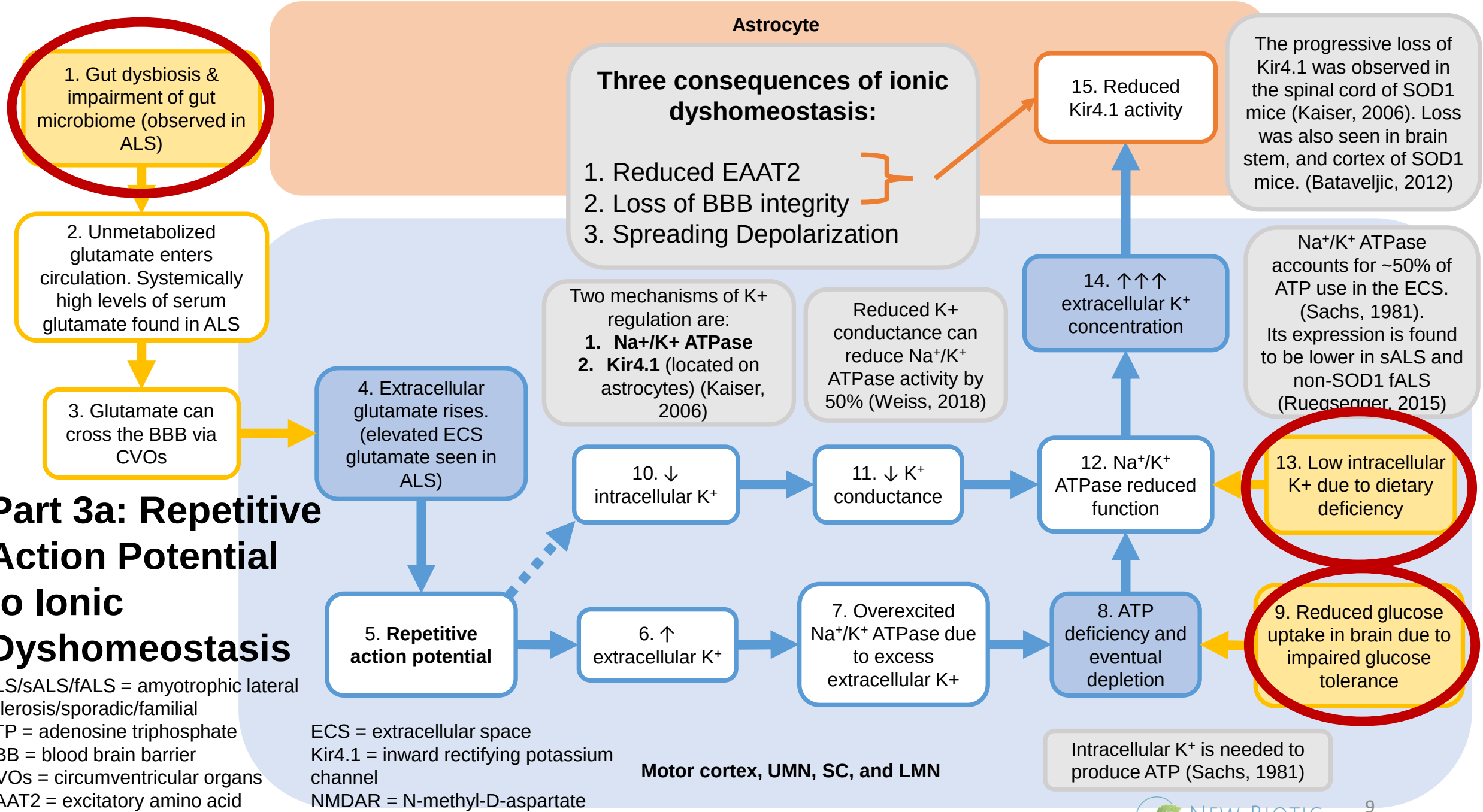
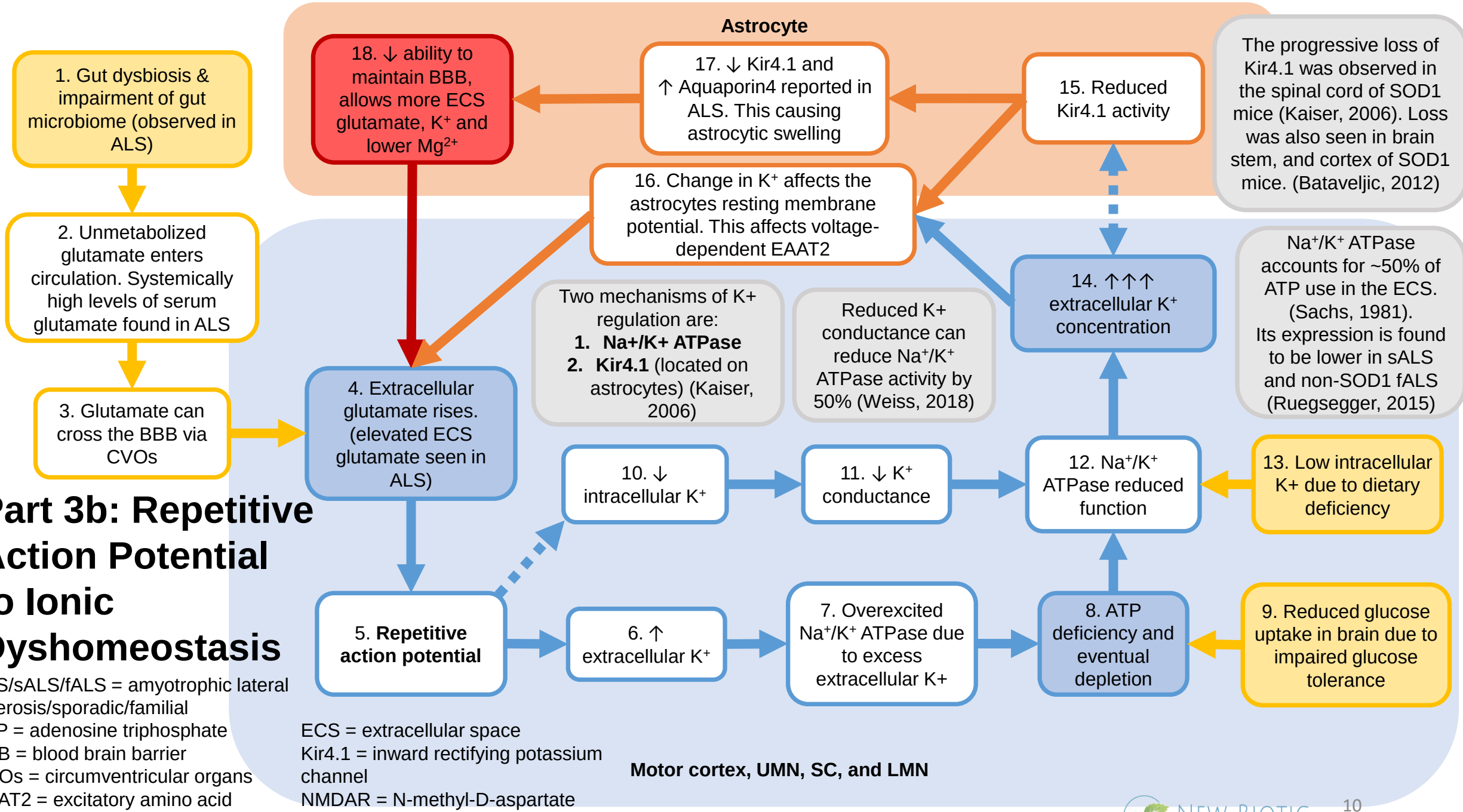
Part 3a: Repetitive Action Potential to Ionic Dyshomeostasis



ALS/sALS/fALS = amyotrophic lateral sclerosis/sporadic/familial
ATP = adenosine triphosphate
BBB = blood brain barrier
CVOs = circumventricular organs
EAAT2 = excitatory amino acid transporter 2

ECS = extracellular space
Kir4.1 = inward rectifying potassium channel
NMDAR = N-methyl-D-aspartate receptor

Part 3b: Repetitive Action Potential to Ionic Dyshomeostasis



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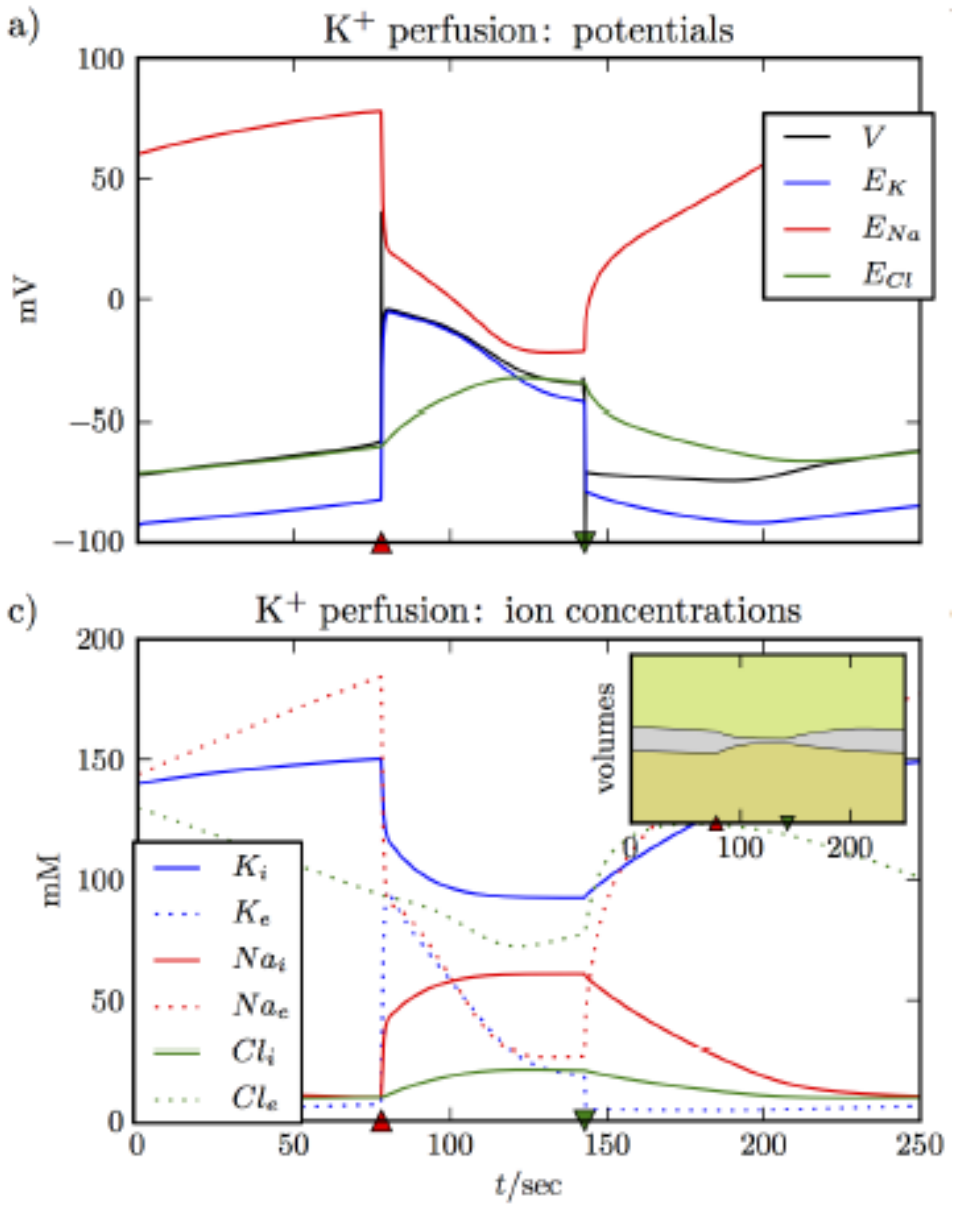
Spreading Depolarization

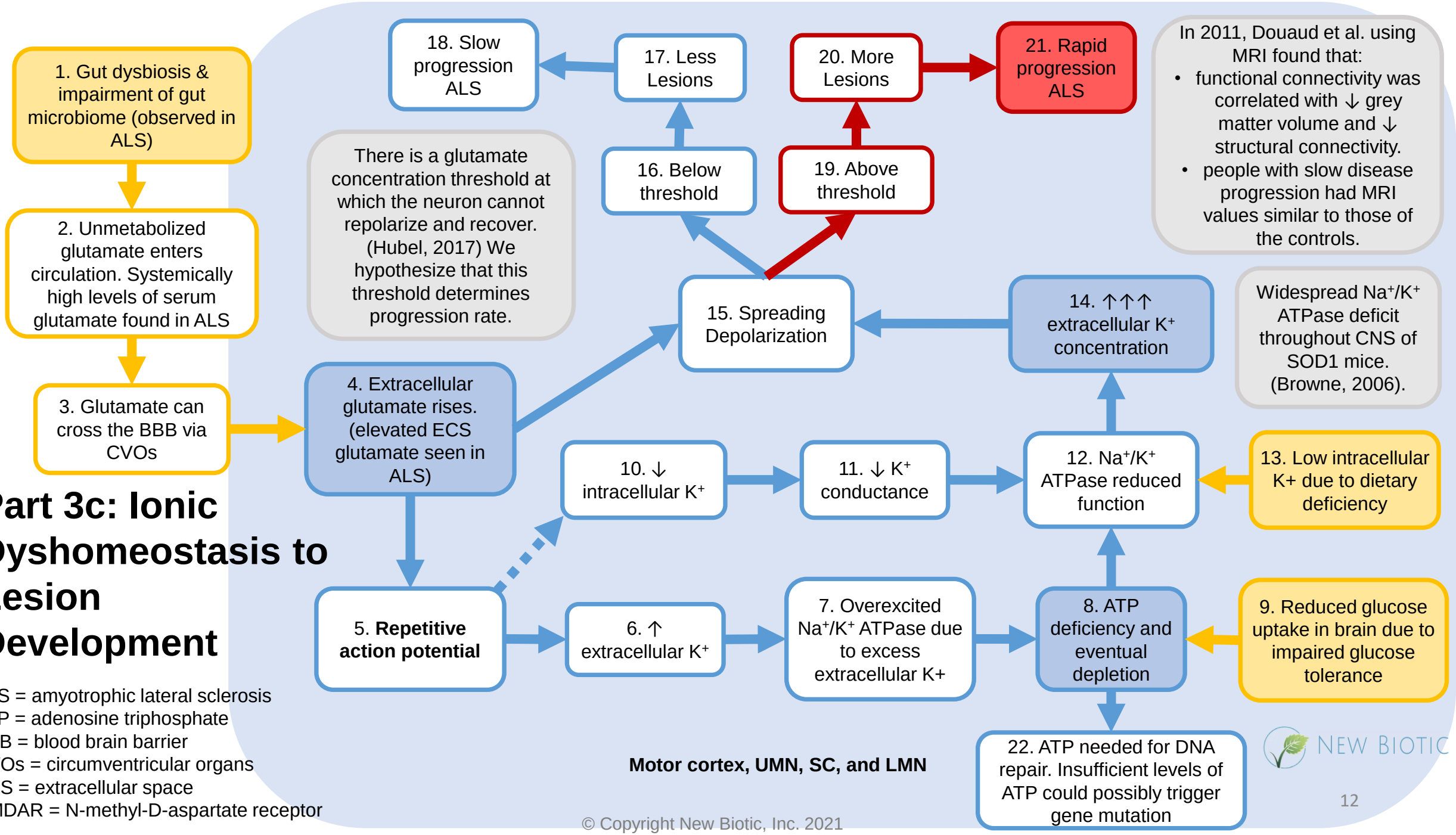
- Spreading depolarization (SD) is a self-propagating wave in which an individual neuron experiences sustained depolarization and delayed recovery.

- Figure on right shows intracellular Na^+ stays high and K^+ stays low for an extended time (~60 seconds). Indicates delayed recovery.

- Caused by K^+ perfusion
- Exacerbated by extracellular glutamate



Part 3c: Ionic Dyshomeostasis to Lesion Development



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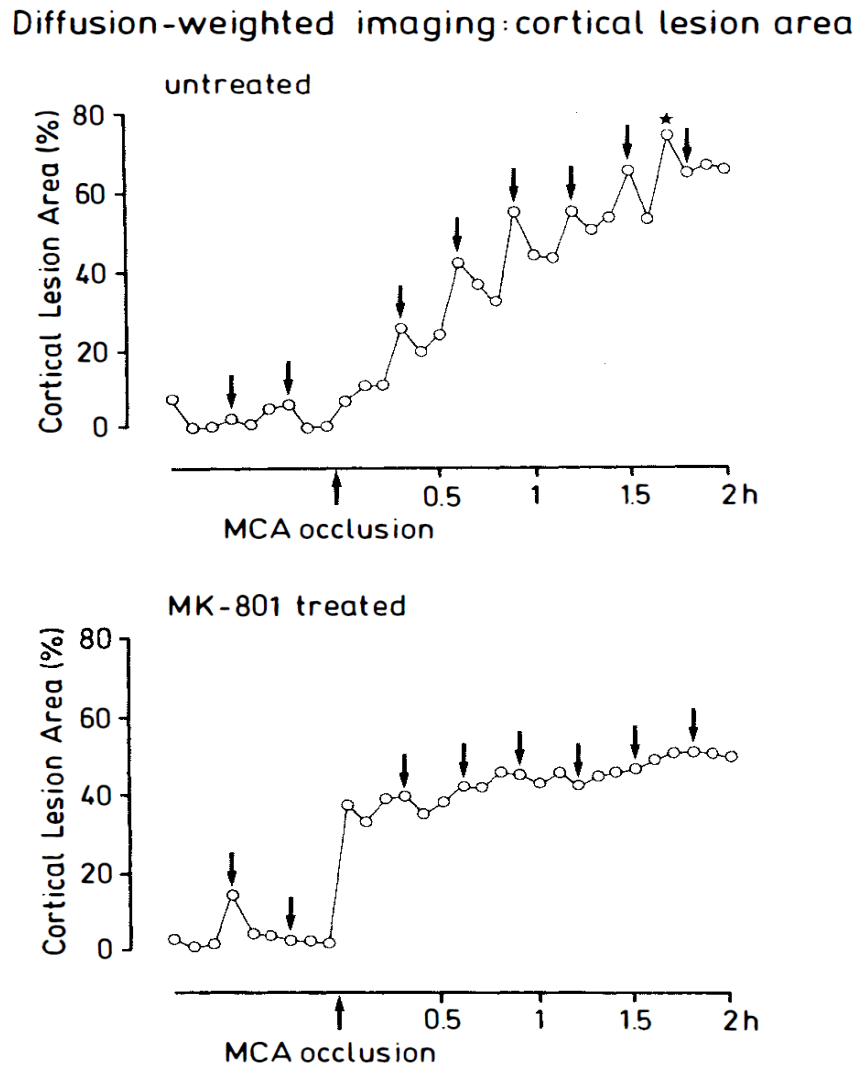
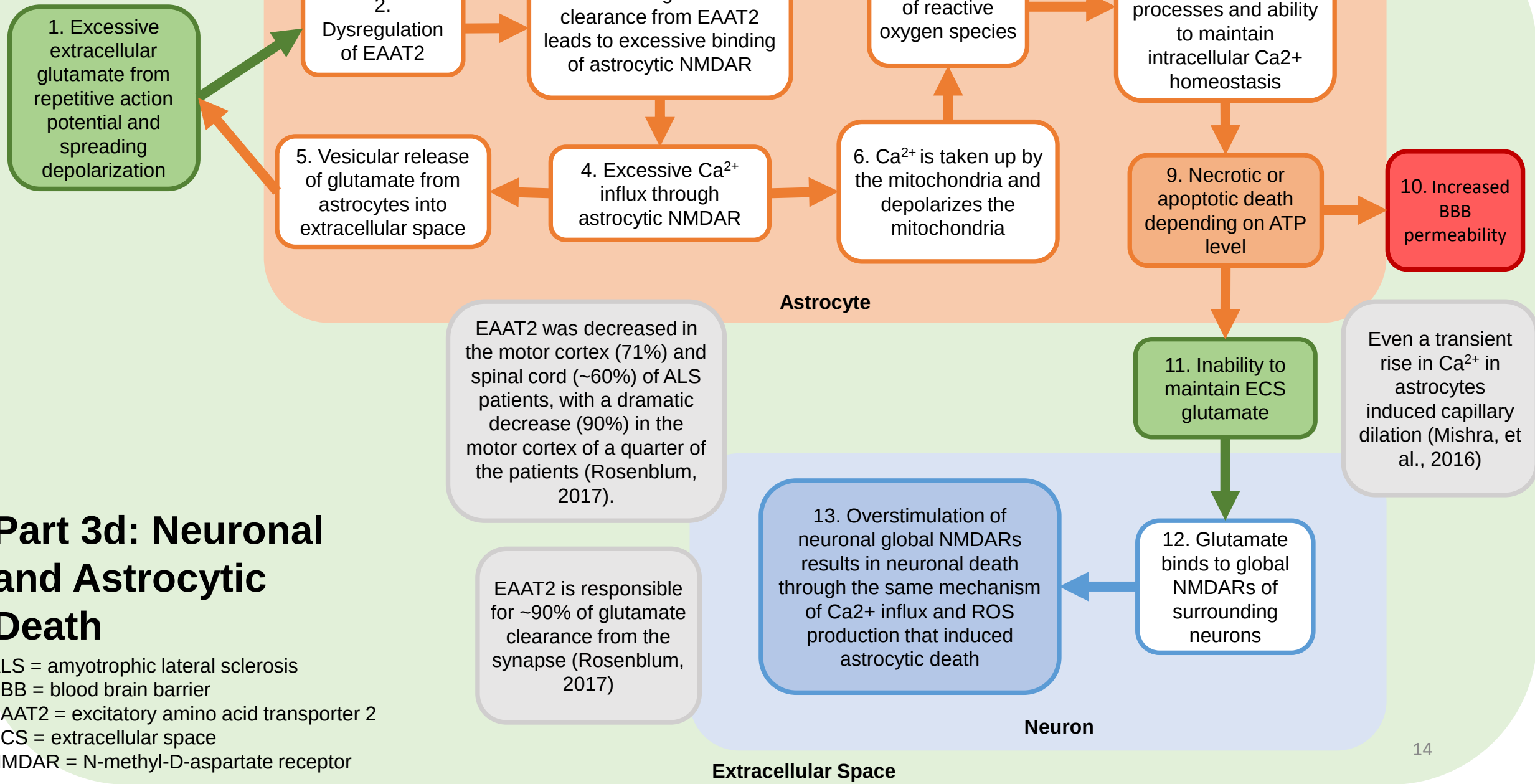
Lesion Development in Spreading Depolarization (SD)

- SD mediates cortical lesion development and secondary brain damages (Hubel, 2017)
- **Busch, 1996:**
  - Microinjections of potassium acetate evoked cortical SD, which led to sharp increase in lesion size. (Figure)
  - NMDAR antagonist (dizocilpine) prevented:
    - ATP depletion
    - K^+-evoked SD
    - Infarct growth in all directions
- Suggests glutamate as the primary/upstream cause of SD and lesions?
- Professor Martin Turner was the first to observe grey and white matter lesions in ALS patients



Part 3d: Neuronal and Astrocytic Death



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Conclusion

- **In summary:**
  - Gut dysbiosis → Glutamate toxicity → repetitive action potential →
    - a) fasciculation → K+ depletion at axon terminals → cramp → metabolic abnormalities → reduced cerebral glucose uptake → reduced ATP generation → ionic dyshomeostasis
    - B) overutilization of Na^+/K^+ ATPase→ ATP deficiency → K+ perfusion → spreading depolarization → lesion

- **This hypothesized pathophysiology suggests:**
  - Gut dysbiosis, dietary potassium deficiency, and metabolic abnormalities may be cause of ALS onset.
  - ALS could be detected early on by examining ionic dyshomeostasis. glutamate toxicity, and earlier (observable) symptoms of ALS
  - ALS heterogeneity may be explained by the extracellular glutamate concentration threshold (with concentrations above the threshold leading to SD lesions and fast progression).

Conclusion

- Currently, diagnostic delay is the main contributor for not being able to treat ALS early. Thus, a new diagnostic protocol is urgently needed to detect ALS at the earliest stage.
- This plausible pathway offers opportunity for a new diagnostic protocol and new ALS treatment at earliest stage before permanent damage to motor neurons (due to dying back process) or ALS gene mutations occur (due to low or depleting ATP level that may indirectly trigger gene mutation).

- **Next steps are to test:**
  - Role of gut dysbiosis/Effect of probiotic treatment in ALS patients
  - Intracellular K^+ and Mg^{2+} in ALS patients
  - Extracellular CNS K^+, Mg^{2+}, and glutamate in ALS patients
  - Serum glutamate and Cr^{3+} in ALS patients

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