Determining localisation of Cx36 in the membrane of GABAergic neurons in relation to aspartate signalling in epileptic seizures

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Key points

Introduction to epilepsy
- prevalence
- causes
- complications
- treatment

Candidate transmitter
- signalling
- accumulation
- transport

Project outline
- laboratory techniques
- future impact

Epilepsy

Summary

Chronic disorder of the brain and nervous system

Characterised by recurrent seizures due to excessive neuronal activity - over-firing of neurons

Seizures vary in duration, behaviour, frequency and affected brain region

- 40 types of seizures identified currently(1)
- classified into 3 major types
  - focal onset (one area/side)
  - generalized onset (both sides)
  - unknown onset
- divided into motor (e.g. tonic-clonic) and non-motor (absent) based on the seizure symptoms(2)

Epilepsy

Prevalence

Affects over 70 million people globally[3], with 633,000 currently living with the disorder in the UK

600 people are diagnosed every week in the UK (1)

Affects individuals of all ages, nationalities and sexes, though incidence is higher among infants, people aged over 50 and in low-income regions (3)

Complications

- seizures can be exhaustive, cause injury and disrupt to daily life
- sudden unexpected death in epilepsy (SUDEP) - nocturnal seizures (4)
- higher prevalence of depression and anxiety disorders (5) - affects almost a third of those with epilepsy (twice the average) (6)

Epilepsy

Causes

There is no one single root cause

Though certain factors can increase likelihood of onset

Such as premature birth, genetic conditions, family history, neurodegenerative disorders, trauma or infection affecting the brain (7)

Genetic epilepsies can be inherited, caused by individual mutations or a symptom of a greater genetic condition

- seizure mechanism remains unclear, so genetic links haven't been established
- one study reviewed 977 genes associated with epilepsy (8)
- one approach is to investigate genes involved in signalling

Epilepsy

Treatment

There is no common cause to target, so the symptoms are treated instead

The most common treatment are antiepileptic drugs (AEDs) that decrease the frequency or severity of seizures

AEDs rebalance the level of excitatory and inhibitory transmission in the nervous system which is disrupted during seizures – where excitation is favoured (9)

Limitations: adverse effects, strict treatment course, drug resistant epilepsy (10)

The resistance to AEDs, involvement of mutations and lack of long-term solutions suggest that genetic therapies are the next step in epilepsy treatment

Aspartate

(also known as aspartic acid)

Summary

excitatory neurotransmitter in GABAergic neurons

Signalling in the CNS is balanced between excitation and inhibition

- main excitatory transmitter - glutamate
- main inhibitory transmitter - GABA

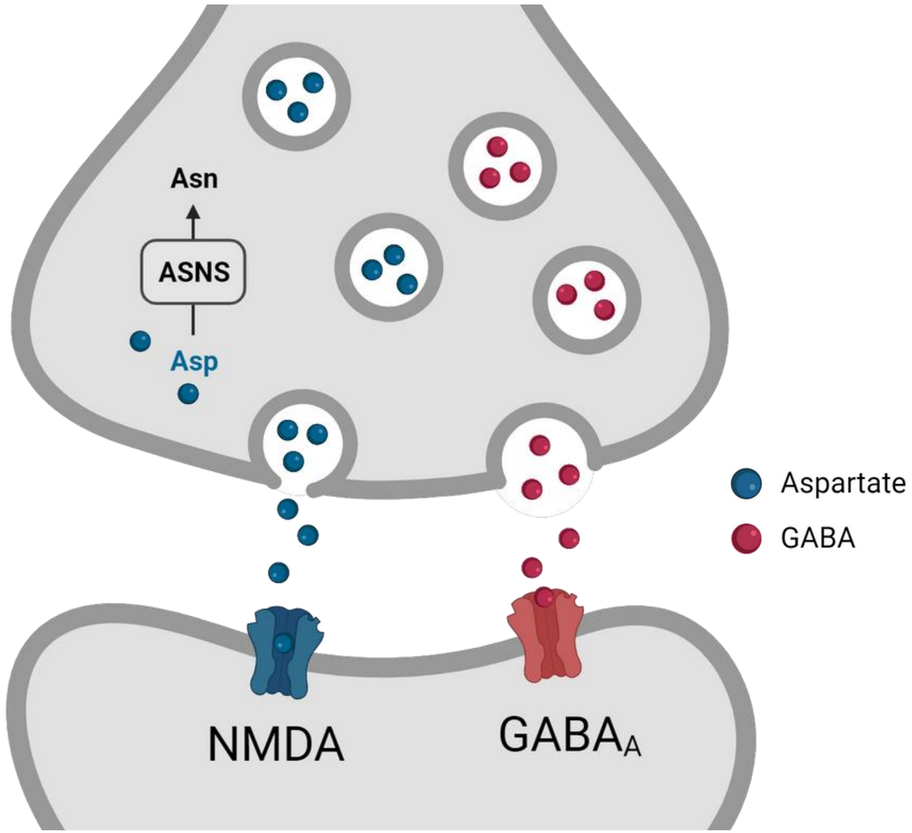
Aspartate acts similarly to glutamate (on NMDA receptors), despite being released from GABAergic neurons

Found to accumulate in the extracellular space, especially when an intracellular conversion enzyme asparagine synthetase (ASNS) is blocked (11)

Excess aspartate can result in seizure-like activity in brain slices and epileptic mouse models (12)

Functional ASNS enzyme

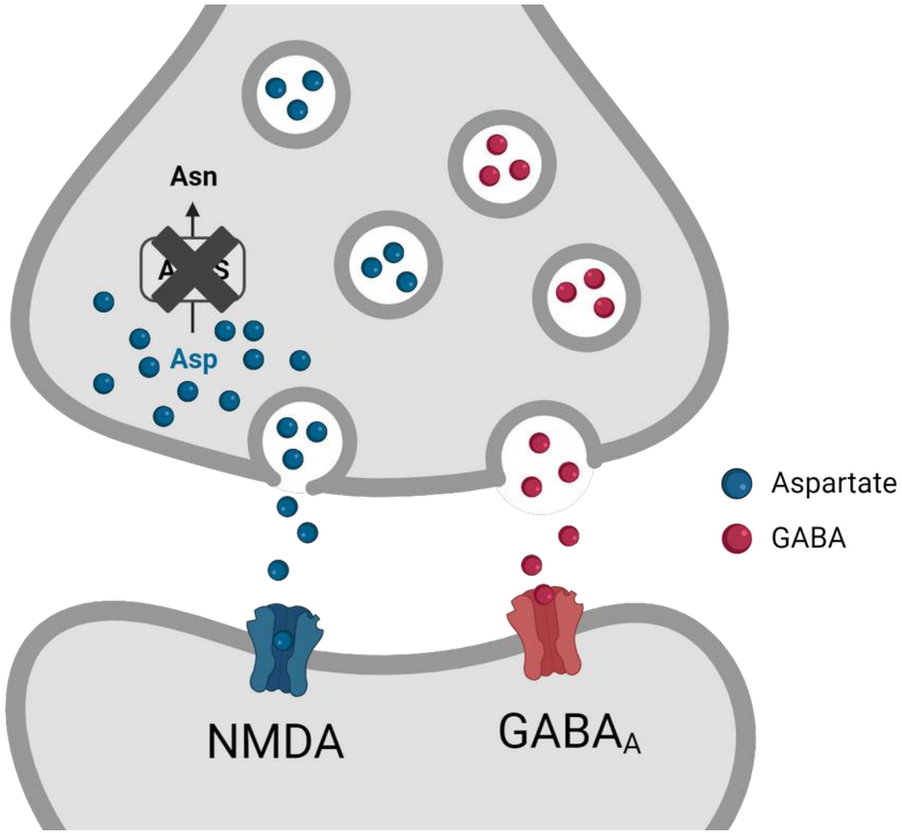
pre-synaptic terminal



post-synaptic terminal

Dysfunctional ASNS enzyme

pre-synaptic terminal



post-synaptic terminal

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Research question

If ASNS dysfunction causes aspartate build up inside the neuron, how has it been found in the extracellular space?

How is the transmitter travelling across the cell membrane?

Suggested solution: an ion channel

An alternative mode of signal transmission has been observed where the transmitter is released through ion channels, rather than vesicular fusion

- present in taste bud cells (CALHM1) as well as some neurons (13)
- released in response to action potential (14)

Connexin 36 (Cx36)

candidate ion channel that is present in the membrane of GABAergic neurons

Cx36 is a connexin expressed in mammalian neurons, forming a gap junction

Gap junctions mediate electrical coupling (signalling) of GABAergic neurons

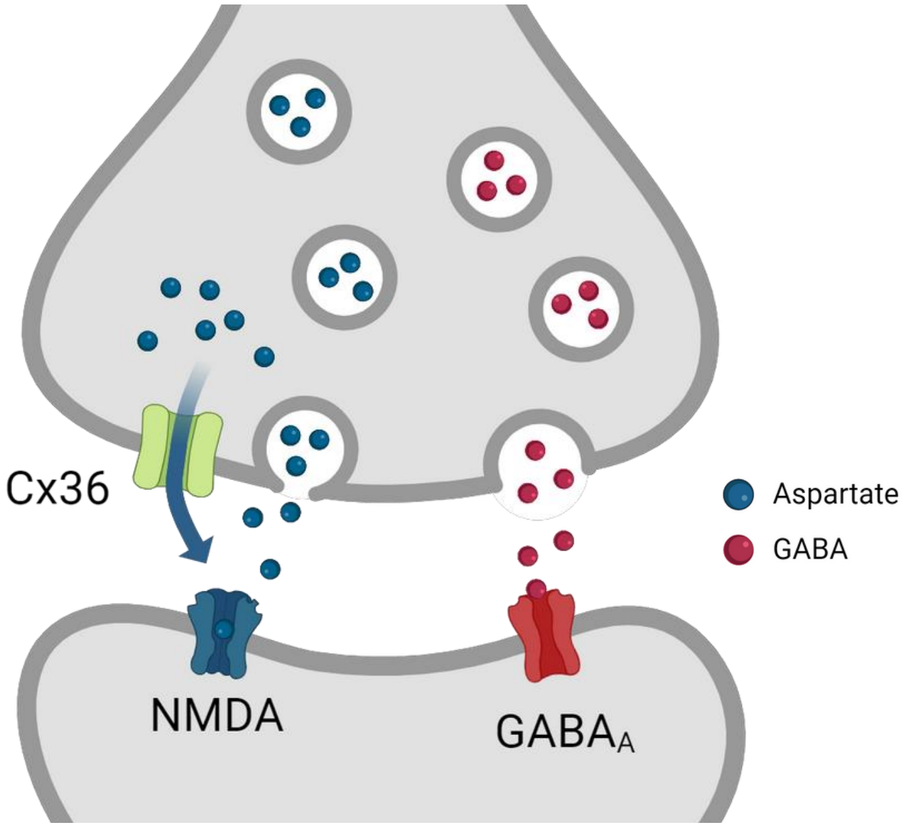
Cx36 deletion → Loss of electrical coupling (15)

Reduced electrical coupling → Reduced seizure action (16)

If Cx36 is the ion channel that facilitates aspartate transport, it will follow one of two models, dependent on its location within the cell membrane

Model 1: Channel synapse

pre-synaptic terminal

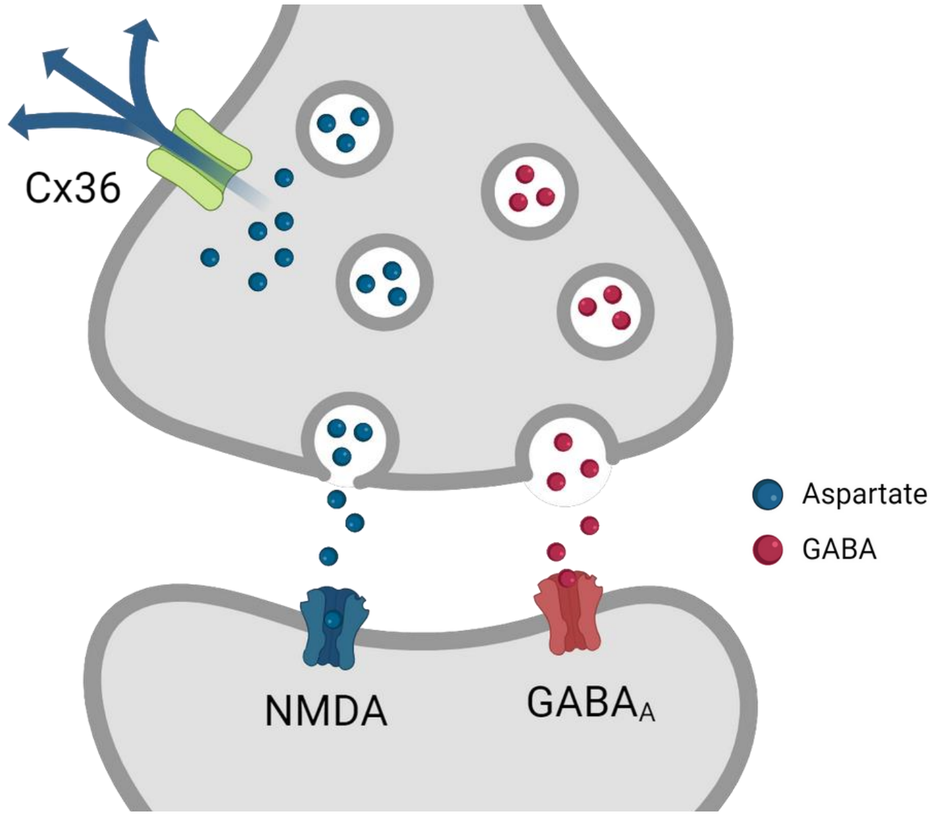


post-synaptic terminal

Specific signal to one cell

Model 2: Volume transmission

pre-synaptic terminal



post-synaptic terminal

Amplified action on surrounding cells

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Project outline

Investigate if Cx36:

- co-localises with ASNS enzyme
- is present at presynaptic terminals
- is orientated towards the synaptic cleft

The findings will indicate which mode of transmission Cx36 is involved in

Techniques to achieve this:

- labelling tissue sections with immunocytochemistry
- confocal and super-resolution microscopy
- quantitative image analysis

Impact

- concluding that Cx36 is involved in aspartate signalling will provide further evidence of the channel's involvement in seizure activity
- study of aspartate signalling offers a new route of treatment for both drugs and genetic therapies
- greater insight into the mechanisms of seizures can allow research into treating the cause, rather than the symptoms

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