Characterization of the elements associated with the synthesis of H_2S in the CNS: expression of CTH in the cortex, striatum and hippocampus

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Introduction

The **deregulation of hydrogen sulfide** (H_2S) levels in the brain seems to be involved in the **origin of several neurodegenerative diseases** (1). However, its precise functions as a gasotransmitter in the central nervous system remains unknown (2) (3). For this reason, the main objective of this study is to **characterize the role of H_2S as a key element in neurotransmission** supporting synaptic plasticity processes in the brain. In this part of the study, we focused in CTH as one of the main enzymes involved in H2S biosynthesis in several organs, but not previusly described in the brain (3).

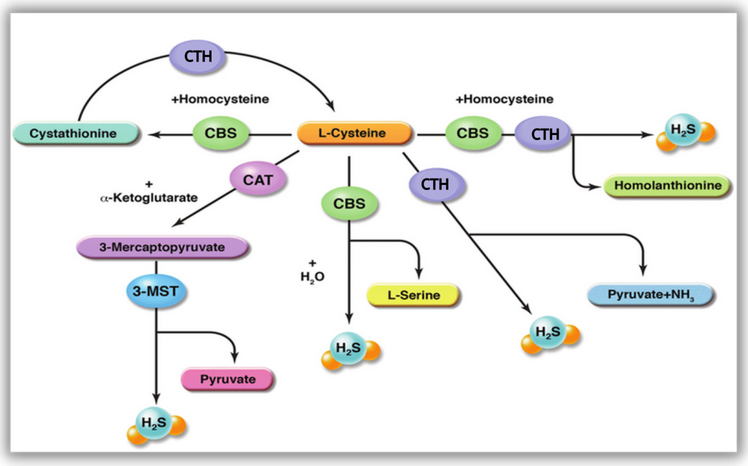

Figure 1. Endogenous biosynthesis of H_2S in mammalian systems. CTH: cystathionine gamma-lyase; **CBS**: cystathionine β synthase ; **3-MST:** 3-mecaptopyruvate sulfurtransferase. Modified from Donnarumma et al., 2017.

Material and methods

Four different 8 weeks-old mice were perfused with 4% PFA. Extracted brain was sectioning with the vibratome with thickness of 30 µm.

We used mouse brain tissue to **characterize the synthesis of H_2S in the brain by immunofluorescence** using a specific antibody for the enzyme CTH. Specifically, sections were subjected to inmmunofluorescence staining with MAP2 (microtubuule-associated protein 2) antybody, neuronal marker; and anti-CTH, anti-Cystathionine γ-lyase antibody.

Results

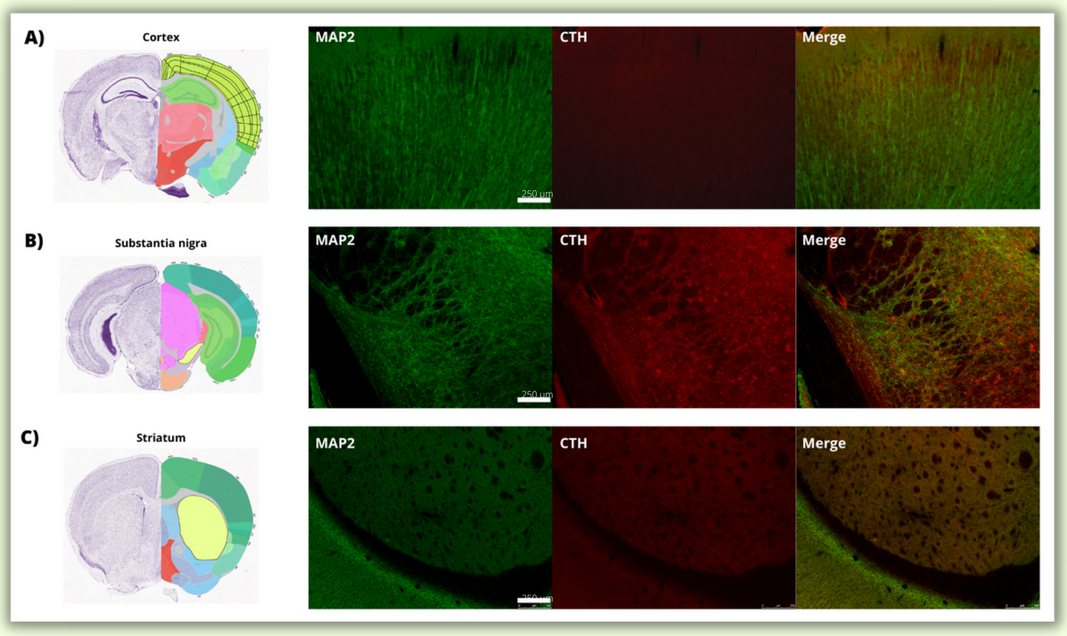

Figure 2. Atlas images of estructures of mouse brain (yellow) and representative images of immunofluorescence staining of mouse brain tissues with MAP2, neuronal marker (green); and anti-CTH (red).

A) Representative image of cerebral cortex. CTH was widely expressed in cortex, evidenced as red staining. Specially, neurons from pyramidal layer showed the highest expression of CTH in both, soma and dendrites, compared to other neuronal populations.

B) Representative image of substancia nigra. Expression of CTH was observed in the two structuctures of substancia nigra, parts compacta and parts reticulata. Neurons from substancia nigra has a prevalence expression of CTH in somas, compared to their projections.

C) Representatiive image of striatum. It has been observed that CTH was specially expressed in somas of medium spiny neurons from striatum tisssue, but with less expression in their projections.

Scale bar: 250 µm
Atlas images were addapted from Allen Mouse Brain Atlas.

Results

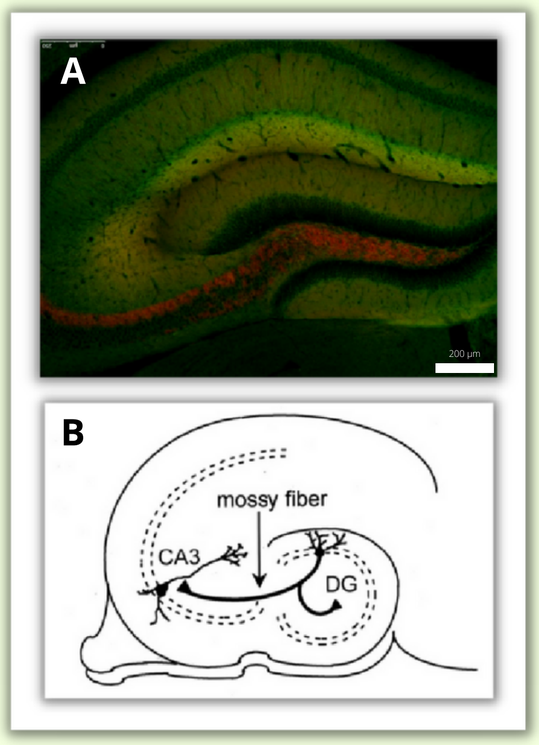

Figure 3. A) Mouse hippocampal region. CTH was expressed in mossy fibers from CA3 to dentate gyrus) evidenced as red staining, indicating a specifiic CTH localization in this specific hippocampal region.

Scale bar: 200 µm

B) Localization of mossy fibers from hippocampal region.
CA3 receives input from mossy fibers of granule cells in DG. Reproduced from Ikegaya, 1999.

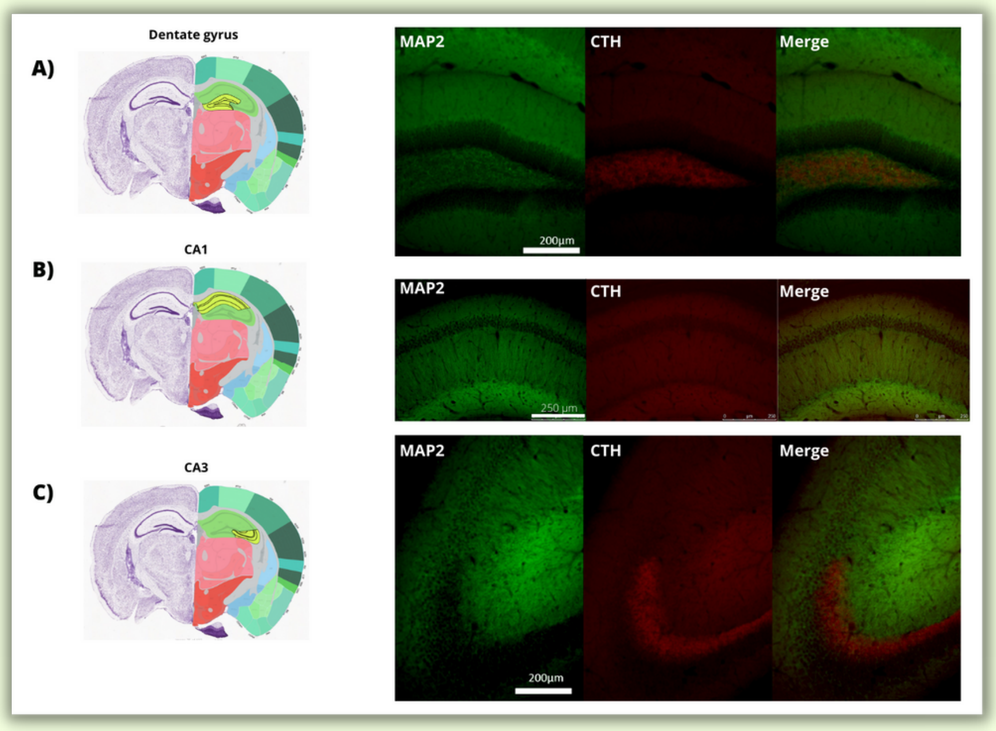

Figure 4. Atlas images of estructures of mouse brain (yellow) and representative images of immunofluorescence staining with MAP2 and anti-CTH antibodies of hippocampal region. Modified from Allen Reference Atlas.

A) **Dentate gyrus (DG)** - CTH was expressed in Hilus. Scale bar: 200µm.

B) **Cornu Ammonis 1 (CA1).** Scale bar: 250 µm.

C) **Cornu Ammonis 3 (CA3) -** CTH has a specifically expression pattern. Scale bar 200 µm.

Conclusion and perspectives

Our study demonstrate an important presence of the enzyme CTH in the brain, an enzyme never described before in this organ. Particularly, we found a specific partern of expression of CTH in DG and CA3 hippocampal regions. CTH is localizated in projections from de granullar cells to CA3, the axons of DGs mossy fibers, but not in somas. The specific presence of H_2S-producing enzyme in the hippocampus suggest that H_2S **has a critical role in memory consolidation**.
Understanding the regulation of the H_2S production and the specific stimuli that induce their release will provide new insights into the biology of H_2S and the **development of novel therapies for neurodegenerative diseases**.

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