

## Functional Analysis of 3-Mercaptopyruvate Sulfurtransferase Using Knockout Mice

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### Abstract

3-mercaptopyruvate sulfurtransferase (MST) transfers sulfur from 3-mercaptopyruvate (3MP) or thiosulfate to a sulfur acceptor. We developed MST-knockout (MST-KO) mice to clarify the physiological role of MST. They showed increased anxiety-like behaviors. In the prefrontal cortex and hippocampus, levels of serotonin (5-hydroxytryptamin, 5-HT) and/or serotonin metabolite, 5-hydroxyindoleacetic acid were higher in MST-KO mice than in wild-type mice and further 5-HT<sub>2A</sub> receptor mRNA was increased. Experiments using MST-KO mouse revealed that MST produces hydrogen polysulfides from 3MP and sodium sulfide and hydrogen trisulfide was also enzymatically produced.

**Keywords:** 3-Mercaptopyruvate sulfurtransferase; Knockout mice; Mercaptolactate-cysteine disulfiduria; Serotonin; 5-Hydroxyindoleacetic acid; Serotonin receptor; Anxiety-like behavior

### Introduction

3-mercaptopyruvate sulfurtransferase (MST) is a universal enzyme in prokaryotes and eukaryotes. In 1998, we reported the distribution of MST in rat [1]. MST was expressed in numerous somatic cells and was found to be present in both cytosol and mitochondria [1] (Figure 1a). In the kidney, MST levels were higher in the cortex and proximal convoluted tubules as compared to that in the medulla and distal convoluted tubules, respectively [1] (Figure 1b). In the liver, MST levels were higher in hepatocytes localized around central veins as compared to that in the portal areas [1] (Figure 1c). In the heart muscle, MSTs were found in the myocardial cells around the perinuclear area [1] (Figure 1d). In the lung, MST levels were higher in the bronchiolar epithelial cells, including ciliated and non-ciliated cells, as compared to that in the alveolar cells [1] (Figure 1e). In the brain, MSTs were present in the stellate-shaped glial cells but not in the neural cells [1] (Figure 1f). In the testis, MSTs were detected in the leydig cells [1].

MST catalyzes the enzymatic transfer of sulfur atoms from either 3-mercaptopyruvate (3MP) or thiosulfate to a sulfur acceptor. Alternatively, MST transfers sulfur to cyanide or to thiols to produce thiocyanate and hydrogen sulfide (H<sub>2</sub>S), respectively [2,3]. Various physiological roles of H<sub>2</sub>S have been reported, including its ability to act as a neurotransmitter. In the hippocampus, H<sub>2</sub>S increases the activity of N-methyl D-aspartate receptors resulting in long-term potentiation, which suggests that H<sub>2</sub>S is an endogenous neuromodulator in the brain [4]. In the vascular system, H<sub>2</sub>S activates potassium channels in the smooth muscles resulting in their relaxation and hyperpolarization of their membrane potential in thoracic aorta, portal vein, and ileum, particularly in association with nitric oxide [5,6]. In astrocytes, H<sub>2</sub>S activates transient receptor potential (TRP) channels, resulting in calcium influx [7].

A defect of MST causes mercaptolactate-cysteine disulfiduria (MCDU) [8], which causes mental retardation in severe MCDU patients [9,10]. However, the pathogenesis of MCDU is not known. To investigate the mechanisms underlying this defect, we recently developed MST-knockout (MST-KO) mice [11]. We report the recent study that compare MST-KO mice with wild-type mice and the study that was carried out in wild-type mouse cells and MST-KO mouse cells.

### The characteristics of MST-KO mice

MST-KO mice demonstrated increased anxiety-like traits in various behavioral tests [11]. In the open field test, MST-KO mice moved less during the first 9 min as compared to wild-type mice [11] (Figure 2a). In the light/dark transition test, as compared with wild-type mice, MST-KO mice spent longer time in the dark chamber as opposed to the light chamber and demonstrated a longer latency of first transition from the dark chamber to the light chamber [11] (Figures 2b and 2c). In the elevated plus-maze test, MST-KO mice tended to spend less time in the open arms and to move shorter distances than wild-type mice [11] (Figure 2d).

In the prefrontal cortex, levels of serotonin (5-hydroxytryptamin, 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA, a metabolite of 5-HT) in MST-KO mice were higher than those in wild-type mice [11] (Figure 3a). In the hippocampus, 5-HIAA levels were also increased in MST-KO mice as compared to wild-type mice but the 5-HT levels did not differ [11] (Figure 3b). It is reasonable that expression of 5-HT<sub>2A</sub> receptor mRNA was increased in MST-KO mice as compared to wild-type mice [12] (Figures 3c and 3d). Because MCDU has been reported to be associated with mental retardation in humans [9,10], MST defects may have a connection with depression, although this has not been proven.

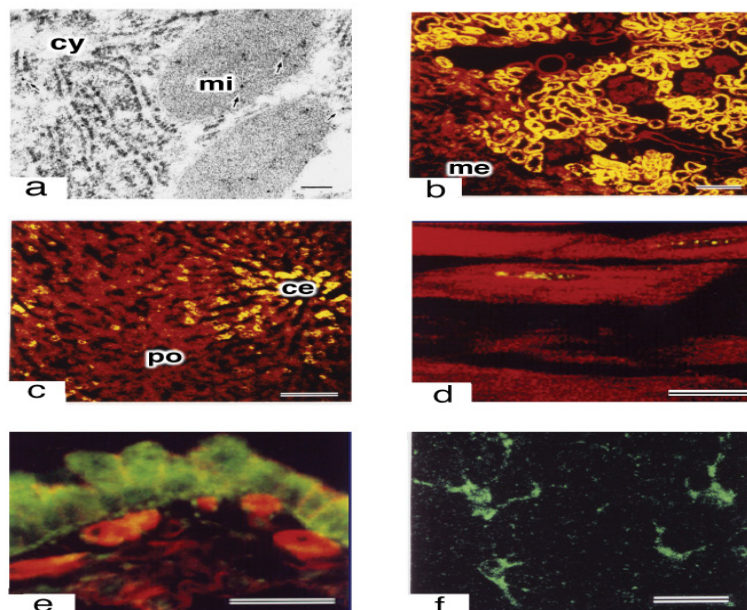
No morphological or histological differences were observed between MST-KO and wild-type mice [11]. Hematoxylin and eosin and Nissl staining of the cerebrum and cerebellum did not reveal any histological differences between MST-KO and wild-type mice (Figure 4). Similarly, hematoxylin and eosin staining of liver and kidney did not reveal any difference between MST-KO and wild-type mice [11]. Although MST-KO mice developed normally and did not appear to have anomalies [11], further investigation is necessary.

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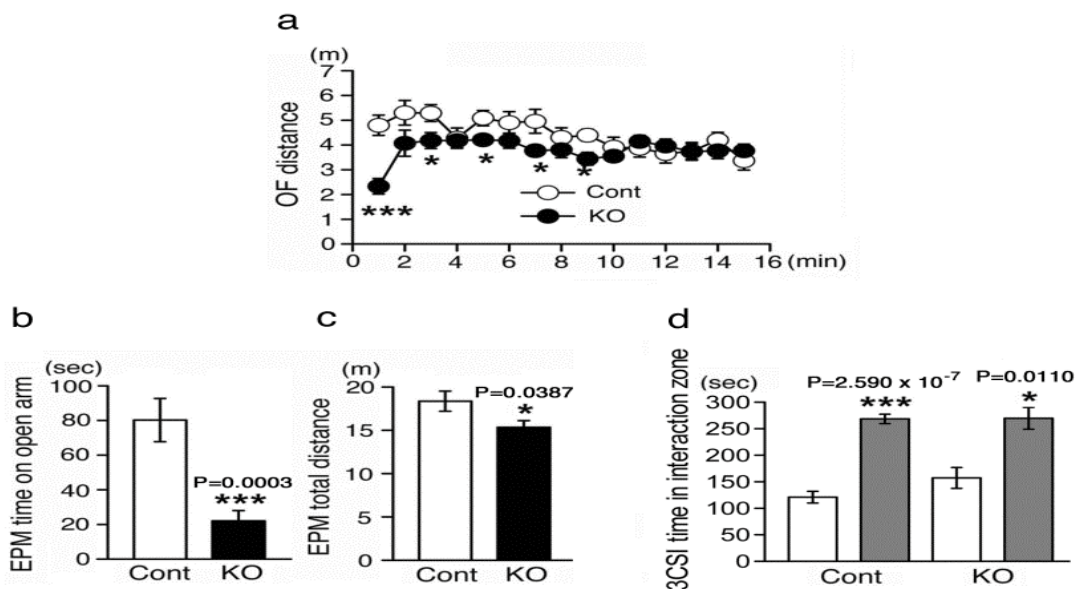
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**Figure 1:** Distribution of MST.

(a) Immunoelectron microscopic analysis of the hepatocyte. Gold particles (arrows) were detected on mitochondria (mi) and were scattered in the cytoplasmic matrix (cy). (b-f) Immunohistochemical staining of using a confocal laser scanning microscopy. (b) In the kidney, the cortical area was stained strongly compared to the medulla (me). (c) In the liver, staining is stronger around the central area (ce) as compared to that around the portal area (po). (d) In the heart, MSTs were detected around the perinuclear area. (e) In the lung, MSTs were detected diffusely in the cytoplasm of bronchiolar epithelial cells. (f) In the brain, MSTs were detected in the stellate-shaped glial cells. Bars a 0.1  $\mu\text{m}$ , b, c 100  $\mu\text{m}$ , d-f 20  $\mu\text{m}$ . (Figures 3A, 3C, 3E, 3F, 3G and 4B from Nagahara et al. [1].)



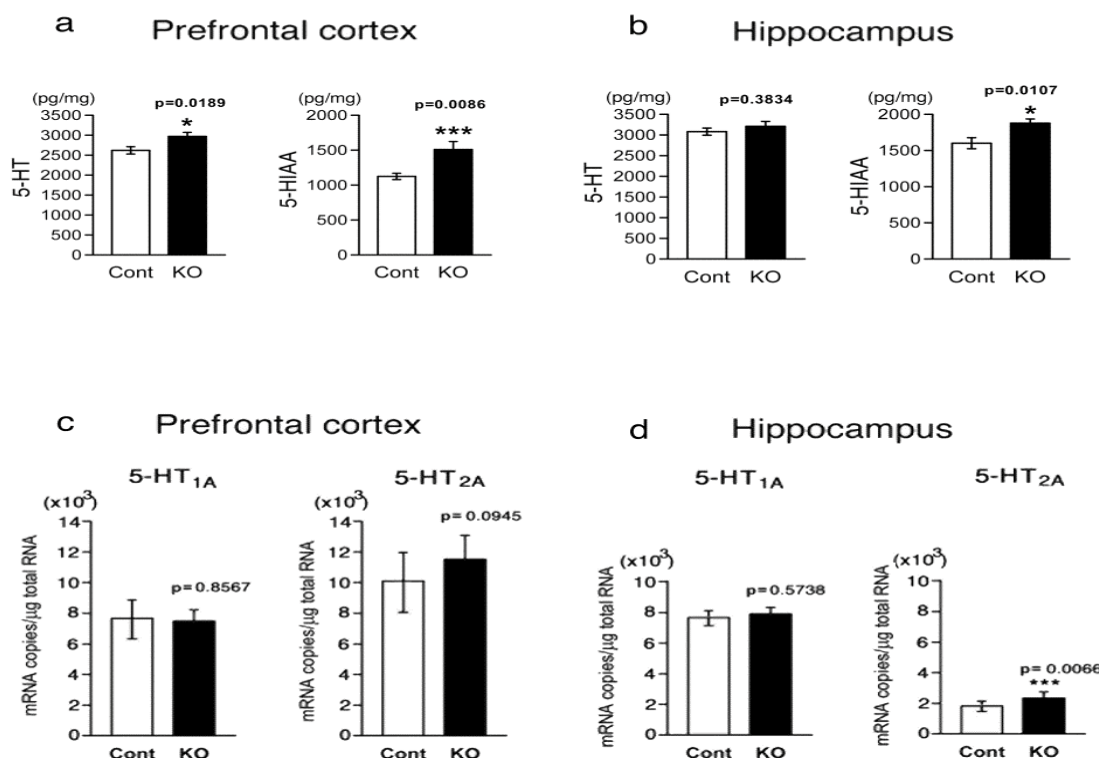
**Figure 2:** Behavioral tests.

Behavioral tests between MST-KO mice and wild-type mice. (a) Open field test (OF). P values of data from 1 to 15 min between MST-KO and wild-type groups are  $3.851 \times 10^{-5}$ , 0.1001, 0.0240, 0.8534, 0.0290, 0.1822, 0.0373, 0.3342, 0.0113, 0.4355, 0.500, 0.5284, 0.9876, 0.3265, and 0.3905, respectively). (b, c) Elevated plus-maze test (EPM). Details are given in the text. (d) 3-Chambered social interaction tests (3CSI). The values of white bars represent the interaction time with empty cage. The values of gray bars represent the interaction time with the cage in which a stranger mouse resides. All data represent the mean  $\pm$  S.E.M (bar). \*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ ; Cont, wild-type mice; KO, homozygous MST-KO mice. (Figures 3d, 3g, 3h and 3i from Nagahara et al [11].)

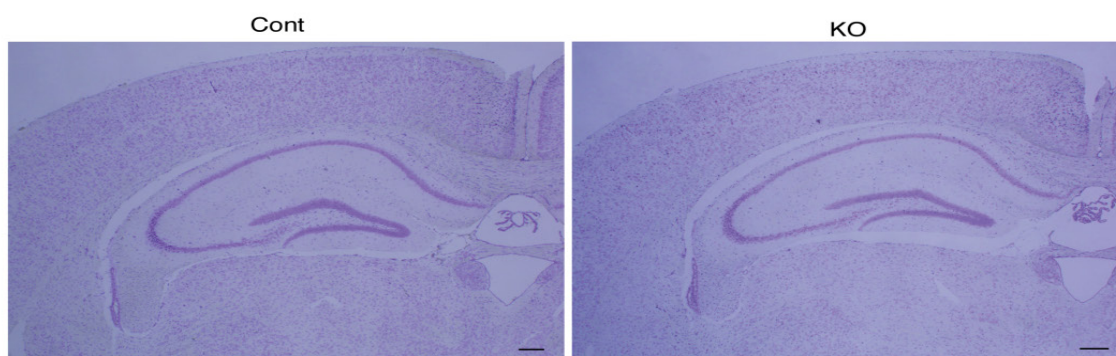
## Determinations of polysulfide-producing enzymes

Experiments using MST-KO and wild-type mouse brain cells demonstrated that MST produces hydrogen polysulfide (H<sub>2</sub>Sn) [13]. H<sub>2</sub>Sn has a greater ability to activate TRP channels in astrocytes than

H<sub>2</sub>S [14] and has been reported to activate TRP ankyrin 1 channels in sensory neurons [14,15], leading to acute pain [15]. In addition, H<sub>2</sub>Sn induces kelch-like ECH-associated protein 1 to form oxidized dimers and nuclear factor-like 2 to translocate to the nucleus [16]. In Neuro2A cells, H<sub>2</sub>Sn increases Akt phosphorylation and may protect cells from



**Figure 3:** Monoamines analyses and quantitative analyses of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor mRNAs. Regional brain monoamines in MST-KO mice were assayed. (a) Prefrontal cortex. (b) Hippocampus. Monoamine contents were expressed in pg monoamine per mg tissue protein. (Figures 4b, c f and g from Nagahara et al [11]) Regional brain 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor mRNAs in MST-KO mice were assayed. (c) Prefrontal cortex. (d) Hippocampus. Amount of 5-HT<sub>1A</sub> receptor mRNA was expressed in copies per  $\mu$ g tissue total RNA. (Figure 15 from Nagahara et al [12].) All data represent the mean  $\pm$  S.E.M (bar). \*, p<0.05; \*\*\*, p<0.001; Cont, control mice; KO, homozygous (null) KO mice.



**Figure 4:** Morphological findings of the hippocampus in the brain. Nissl staining for coronal sections of the hippocampal areas of wild-type and the mutant adult brains. Cont, control mice; KO, MST-KO mice. Bars, 200  $\mu$ m. (Figure 2d from Nagahara et al. [11].)

oxidative damage [16] while also functioning to inhibit cell growth and the promotion of neurite outgrowth [17]. In addition, H<sub>2</sub>Sn oxidatively modifies phosphatase and tensin homolog in intact cells [18]. Moreover, it has been reported that H<sub>2</sub>Sn promotes protein kinase G Ia to form the active homodimer and induces vasodilation [19].

Hysin and Wood suggested that enzymes from rat liver produced polysulfides from 3MP in 1959 [20]. In 2015, Kimura et al. confirmed that MST produced hydrogen trisulfide (H<sub>2</sub>S<sub>3</sub>) and H<sub>2</sub>S from 3MP in brain cells [13]. Wild-type mouse brain cells produced greater H<sub>2</sub>S<sub>3</sub> and H<sub>2</sub>S from 3MP than MST-KO mice [13]. They also reported that

MST produced hydrogen disulfide (H<sub>2</sub>S<sub>2</sub>), as opposed to hydrogen pentasulfide (H<sub>2</sub>S<sub>5</sub>), from 3MP in brain cells [13]. When exposed to 3MP, levels of H<sub>2</sub>S<sub>2</sub> in wild-type mouse brain cells increased but those of H<sub>2</sub>S<sub>5</sub> did not, whereas the exposure of 3MP did not affect levels of either H<sub>2</sub>S<sub>2</sub> or H<sub>2</sub>S<sub>5</sub> in MST-KO mouse brain cells [13]. In addition, Kimura et al. found that MST and its homolog rhodanese produced H<sub>2</sub>S<sub>3</sub> from H<sub>2</sub>S in brain cells [13]. Both wild-type and MST-KO mouse cells produced H<sub>2</sub>S<sub>3</sub> by sodium sulfide exposure with MST-KO mouse cells producing approximately two-thirds of that produced by wild-type mouse cells, suggesting that other enzymes may produce H<sub>2</sub>S<sub>3</sub> [13]. This was subsequently confirmed by rhodanese mutant experiments [13].

In conclusion, comprehensive and systematic research using MST-KO mice is ongoing with the hope of better understanding the multiple roles of MST in the near future.

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