

1. Overview

This manuscript presents a CMOS-based implantable multi-ion image sensor designed to measure magnesium (Mg^{2+}) and calcium (Ca^{2+}) ions in the brain. The sensor is built with Mg^{2+} - and Ca^{2+} -sensitive membranes to allow selective detection of both ions, aiming to overcome the low selectivity of Mg^{2+} sensors for Ca^{2+} . The study includes sensor fabrication, validation experiments, and real-time imaging of ion concentration changes.

This work is potentially significant for neurophysiology and neurological disease research, as Mg^{2+} has been linked to memory and cognitive function. If successful, the sensor could be useful for studying extracellular ion dynamics in the brain. However, several major concerns raise questions about its practical applicability and scientific rigor. These concerns include the sensor's ability to detect physiologically relevant Mg^{2+} levels, the validity of the experimental results due to a lack of statistical analysis, and uncertainties in how spatial and temporal ion distributions are distinguished.

Major Concerns

1. The Sensor May Not Detect Physiological Mg^{2+} Levels: The expected extracellular Mg^{2+} concentration in the brain is ~ 1 mM, but the sensor shows almost no response until significantly higher concentrations (Fig. 7a). This suggests that the limit of detection (LOD) is insufficient, meaning the sensor may not be useful for tracking real biological Mg^{2+} fluctuations. The authors must explicitly determine and report the LOD for Mg^{2+} and test the sensor's ability to detect subtle ($\sim 15\%$) physiological changes.
2. The authors should add justification for the experimental ion concentrations. Figures 5 and 6 use Mg^{2+} and Ca^{2+} concentrations that may not be physiologically relevant, but no justification is given for these choices. The authors should explain how these concentrations were selected and whether they match in vivo conditions. A direct comparison to cerebrospinal fluid composition would help validate the sensor's usefulness.
3. The manuscript does not state how many times each experiment was performed, raising concerns about data reproducibility. There are no error bars in Figures 5-7. Statistical analyses should be performed to validate findings.
4. The manuscript reports $R^2 = 1$ for Mg^{2+} response, but the plot only contains two data points, making this value meaningless. The authors must either include more data points or remove the misleading R^2 value.
5. The sensor covers a relatively wide area, raising concerns about how Mg^{2+} and Ca^{2+} signals are spatially separated. If Mg^{2+} is changing in one part of the sensor and Ca^{2+} in another, how do we ensure correct ion attribution?
6. The manuscript does not specify where the sensor would be implanted or why this particular brain region was chosen and its relevance to Mg^{2+} research.
7. The conclusions overstate the sensor's capabilities without addressing its weaknesses. The manuscript claims "reasonable sensitivity", but the data suggest poor response at biologically relevant Mg^{2+} levels.

Minor Concerns

1. Unclear Terminology and Wording Issues

- Line 39-40: The phrase "aging rate" is unclear. Does this mean the percentage of elderly people or the rate of increase?
- Line 43-44: The manuscript suggests that Mg^{2+} impairs attention and memory, but this is misleading. Dementia impairs these functions, not Mg^{2+} itself.

- Line 56-57: Uses quoted phrases but does not provide references.
- Line 60-69: The manuscript inconsistently switches between "we" and "they", making it unclear whether the authors are referring to their own work or others'.
- Line 91 (Table 1 reference): Table 1 does not actually indicate sensor performance, as claimed.
- Line 214-215: The manuscript states that V_{out} is unaffected by several ions, but also that it does not respond well to Mg^{2+} at lower concentrations, contradicting the claim of good sensitivity.
- Line 261: References "the previous chapter", which is incorrect in a journal article format. It should refer to a specific section instead.

2. Figures Need Improvement

- Figures 5-7 need error bars to show measurement uncertainty.
- Figure 7a only has two data points but reports $R^2 = 1$, which is invalid.
- Figure 8b uses a 0.6V scale for color mapping—how does this relate to real ion concentrations?
- The authors should ensure that all figures accurately reflect the data and support their claims.