2022

Exploring the effects of β-N-methylamino-L-alanine on Schmidtea mediterranea's GluN1/GluN2A NMDA receptor subunits.

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Exploring the effects of β-*N*-methylamino-L-alanine on *Schmidtea mediterranea's* GluN1/GluN2A NMDA receptor subunits.

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Abstract

β-*N*-methylamino-L-alanine, also known as BMAA, is a neurotoxin produced by several species of cyanobacteria and eukaryotic microorganisms such as diatoms and dinoflagellates. BMAA has been implicated in neurodegenerative diseases such as ALS, Parkinson's disease, and dementia. Being that it biomagnifies through the food chain, accumulation occurs in its symbionts like cycad seeds and seafood, and eventually gets ingested by humans causing possible neuronal consequences. There is evidence that BMAA agonizes N-methyl-D-aspartate (NMDA) receptors, a family of L-glutamate receptors that play a critical role in learning and memory, inducing excitotoxicity against neurons. However, exactly how it causes death of motor neurons and how its actions may interact with other neurotoxins or pathological conditions, is not well understood. The focus of this study is to explore planarians *(Schmidtea mediterranea*) as a suitable model system to study the effects of BMAA. Because planarians contain endogenous glutamate and express genes for glutamate receptors, they are a desirable model for investigating a role for NDMA receptors in BMAA exposure. The present study focused on the following hypothesis (1) planarians are a suitable model system to study the effects of BMAA (2) NMDA receptor agonism through BMAA and ethanol exposure induces motor neuronal damage/death in planarians.

Approach

Planarians, *Schmidtea mediterranea*, were incubated in BMAA solutions ranging from 0.80 mM to 0.50 mM for 96 hours. The BMAA solution was removed and replaced with 2% ethanol. Ethanol disrupts cilia and sensitizes the planarians to have to use neuromuscular contraction for movement to discover any dysfunction of motor neurons.

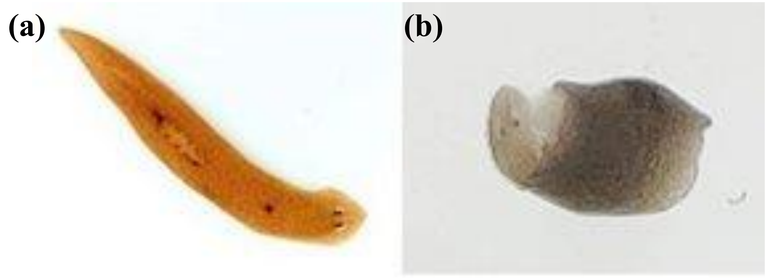

Figure 1. BMAA exposure causes movement defects in planarians. (a) Control planarian in planarian salts with no visible contorting. (b) Planarian after 96 hours of 0.50 mM BMAA exposure in 2% ethanol solution. Contorting and c-shaped curling is very prominent. Movement was only visible in most anterior region of the planarian. In conclusion, long-term exposure to BMAA resulted in a phenotypic expression of contorting and c-shaped curling when cilia were disrupted with ethanol.

RNA interference of *Smed-rootletin*

While we had used ethanol to chemically disrupt cilia in order to uncover neuromuscular movement defects, ethanol has been reported to upregulate NMDA receptor function.

To show that the movement defects we observed were due to BMAA agonizing NMDA receptors rather than a potential synergy between ethanol and BMAA, we knocked down the core ciliary gene, *Smed-rootletin*, to induce inch-worming movement and examine the effect of exposure to BMAA or ethanol.

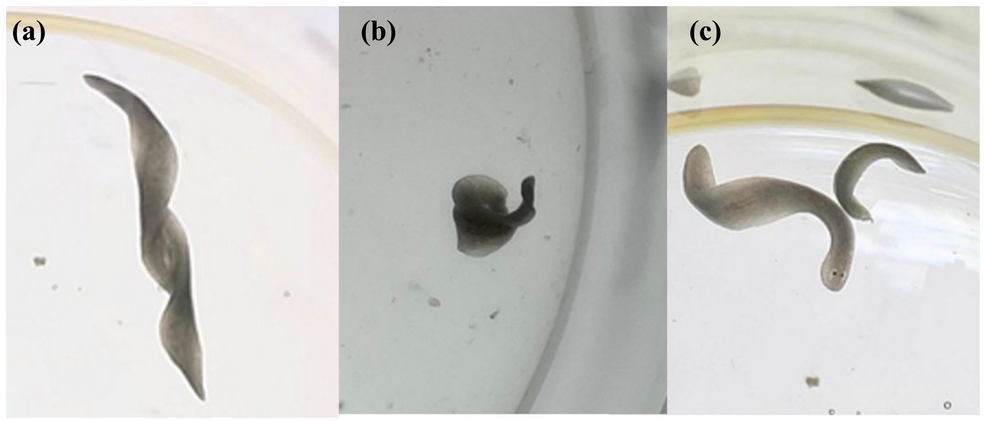

Figure 2. Movement defects due to BMAA or ethanol treatment when ciliary function is disrupted. (a) *Smed-rootletin* RNAi planarian in 0.25mM and (b) 0.5mM BMAA for 48 hours. (c) *Smed-rootletin* RNAi planarian in 2% ethanol for 48 hours.

Both BMAA and ethanol exposure caused c-shaped bending and contorting in the *Smed-rootletin* knockdowns. Similar to what we observed in the BMAA and ethanol exposure. Suggesting that both BMAA and ethanol could be functioning through a similar mechanism to disrupt movement.

Finding planarian NMDA receptor homologs

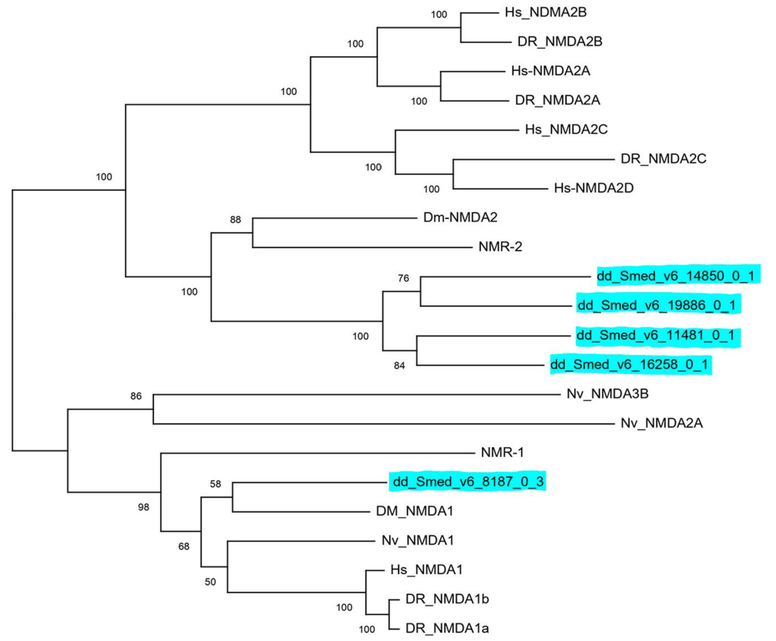

Figure 3. Planarians have NMDA2 homologs. Parsimony tree of NMDA receptors from different organisms: *Homo sapiens* (Hs), *Danio rerio* (DR), *Drosophila melanogaster* (Dm), *Nematostella vectensis* (Nv), and *Schmidtea mediterranea* (dd_Smed_v6). The parsimony tree shows an evident divergence between the NMDA1 and NMDA2 sequences of different organisms. Dd_Smed_v6_8187_0_3 lies within the NMDA1 cluster, closely to the NMDA1 sequences of other organisms. The planarian sequences coding for the NMDA2 receptor subunits also lie within the NMDA2 cluster, implying there is similarity in the sequences. Overall, there is adequate homology between the planarian NMDA receptor sequences and that of other organisms, specifically *Homo sapiens*. The bootstrap values averaging ~90 gives high confidence of the relationships within the tree.

RNA interference of planarian NMDA receptor subunits– Future Directions

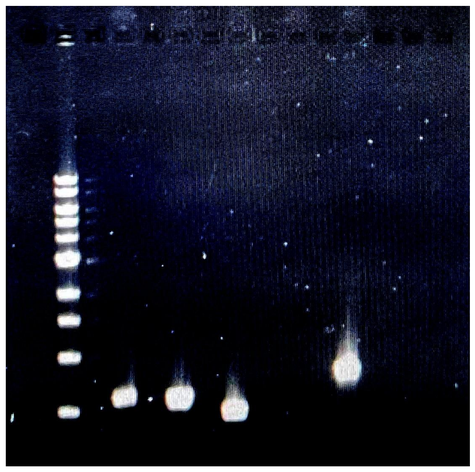

Figure 4. Successful amplification of planarian NMDA genes from a cDNA library.

The planarian homologs of NMDA receptor genes were cloned. These clones were made to generate gene-specific dsRNA to knock down gene function using RNA interference.

We expect that silencing NMDA receptors in planarians would obstruct BMAA's target and serve as protection against its neurodegenerative effects. Therefore, the c-shaped contorting phenotype should not be observed in these knockdowns even in the presence of ethanol and BMAA.

Another important future direction is to focus on which cell types the NMDA genes are expressed in to better understand the function of the NMDA receptors and the specific effects that BMAA or ethanol has on them. This would be accomplished through in situ hybridization with riboprobes made from the clones that I generated from the planarian sequences.

References

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Conclusion

Planarians manifested to be a suitable model system to study the effects of BMAA and ethanol on the homologous NMDA receptor. Bioinformatics and sequence alignments demonstrated adequate similarity between the mammalian NMDA receptor gene and the planarian presumptive NMDA receptor genes- giving the green light for future experiments with such planarian genes. It appears that ethanol and BMAA are both functioning through a similar mechanism that could potentially be an agonistic effect upon the NMDA receptors, inducing what seems like neurodegenerative consequences in the organism based on the contorting phenotype.