Glyphosate infiltrates the brain and may be a risk factor for Alzheimer's Disease

[1,2]Joanna Winstone, [3]Khyatiben V. Pathak, [3]Ritin Sharma, [1]Megan Donnay, [4]Matthew Huentelman, [3]Patrick Pirrotte, [1,2]Ramon Velazquez

1. ASU-Banner Neurodegenerative Disease Research Center, Tempe AZ
2. School of Life Sciences, Arizona State University, Tempe, AZ
3. Collaborative Center for Translational Mass Spectrometry, Translational Genomics Research Institute (TGen), Phoenix, AZ
4. Neurobehavioral Research Unit, TGen, Phoenix AZ

BACKGROUND

- Millions of people around the globe come into contact with toxic pesticides, however the lifelong complications of chronic exposure remain largely unknown (Collotta et al., 2013).
- Agricultural communities, in particular, are at high risk for exposure given the widespread application of pesticides to crops.
- Glyphosate, the active ingredient in RoundUp, has been the most heavily applied herbicide in the U.S. since its inception in the 1970s (Benbrook, 2016).

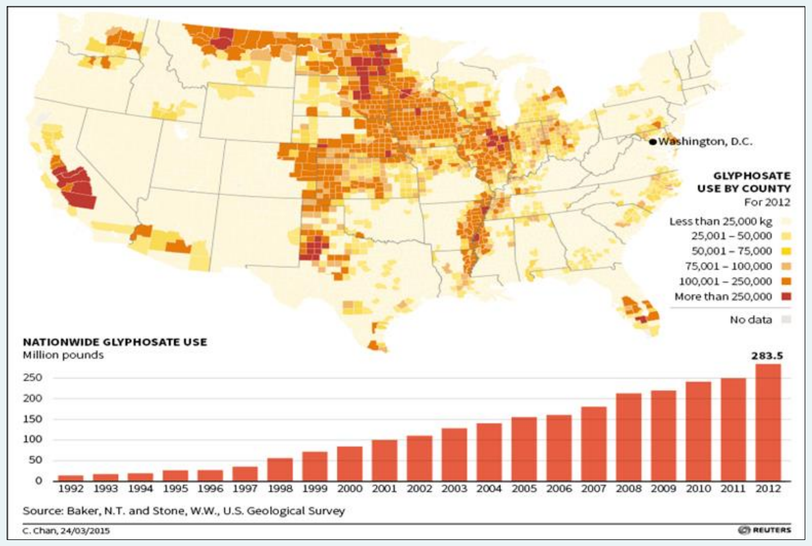


Figure 1. Distribution of glyphosate use across the United States and total annual application by year (Baker & Stone, 2015).

- Evidence indicates that Alzheimer's disease (AD), a neurodegenerative disease characterized by a progressive deterioration of cognitive and functional skills, may be associated with exposure to environmental toxins (Bhagyashree et al., 2019; Armstrong, 2019).
- The rise in glyphosate application to corn and soy crops has a strong, positive correlation with the increase in deaths due to Alzheimer's Disease (Figure 2; Seneff et al., 2015).

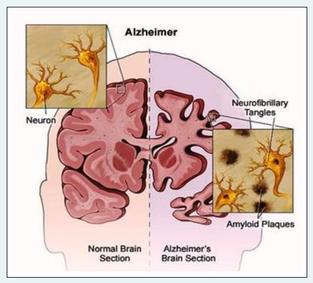


Figure 2. Normal brain vs Alzheimer's brain (Mendoza, 2016). Extracellular plaques made up of amyloid-beta (Aβ) peptides are specific to AD while intracellular neurofibrillary tangles (NFT), comprised of hyperphosphorylated tau protein, accumulate in both AD and other tauopathies (Lebouvier et al., 2017; Querfurth and LaFerla, 2010).

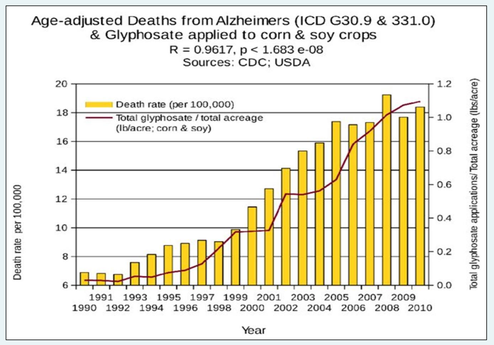


Figure 3. Correlation between deaths due to Alzheimer's disease and glyphosate application to corn and soy crops (Seneff et al., 2015).

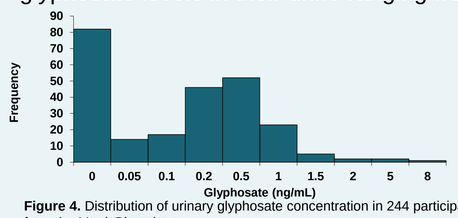
- Since there is an increased prevalence of AD in agricultural communities (Parron et al., 2011) it is critically important to investigate glyphosate exposure as a potential risk factor for AD.

The overall goals of this study were to (1) determine whether glyphosate crosses the blood-brain barrier (BBB) in an *in vivo* model and (2) identify the dose needed in mice to match levels of human exposure. Future work will look at whether glyphosate exposure in an AD mouse model is associated with AD-related pathology and behavioral alterations.

METHODS & MAIN FINDINGS

Determine the distribution of glyphosate levels in human urine.

- We collected preliminary data on glyphosate levels in human urine using the MetricBio Cohort at TGen.
- The MetricBio study consisted of 244 participants from 41 states in the USA (41% male and 59% female, median age of 51 years),
- Of these 244 individuals, over 60% participants had detectable glyphosate levels in their urine ranging from 1-130 µg/L (Figure 4)



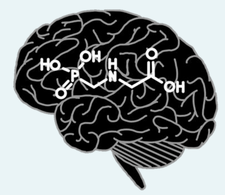


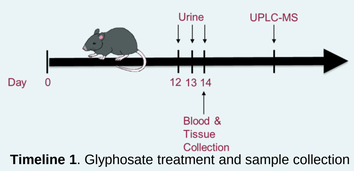
Figure 4. Distribution of urinary glyphosate concentration in 244 participants from the MetricBio cohort.

Demonstrate that glyphosate crosses the BBB in an in vivo model

- Standard lab mice (C57BL/6) were given a dose of either 125, 250, or 500 mg/kg or a vehicle control via daily oral gavage for a total of 2 weeks (Table 1; Timeline 1).
- These doses were chosen based off of previous studies done using 250 and 500 mg/kg to assess the effects of glyphosate on anxiety and depressive like behaviors (Ait-Bali et al., 2017) , with a lower third dose added to create a dose response curve.
- The no observable effect level (NOEL) for glyphosate in mice is set at 500 mg/kg/day (Environmental Protection Agency, 1993).
- Brain tissue was then analyzed in collaboration with the Pirrotte lab at TGen using ultra performance liquid chromatography and mass spectrometry.

Genotype	Gly. dosage	n
C57BL/6	Vehicle	6 (3M + 3F)
C57BL/6	125 mg/kg/day	6 (3M + 3F)
C57BL/6	250 mg/kg/day	6 (3M + 3F)
C57BL/6	500 mg/kg/day	6 (3M + 3F)

Table 1. Dosages for Pilot Study. *Gly. = Glyphosate*



Timeline 1. Glyphosate treatment and sample collection

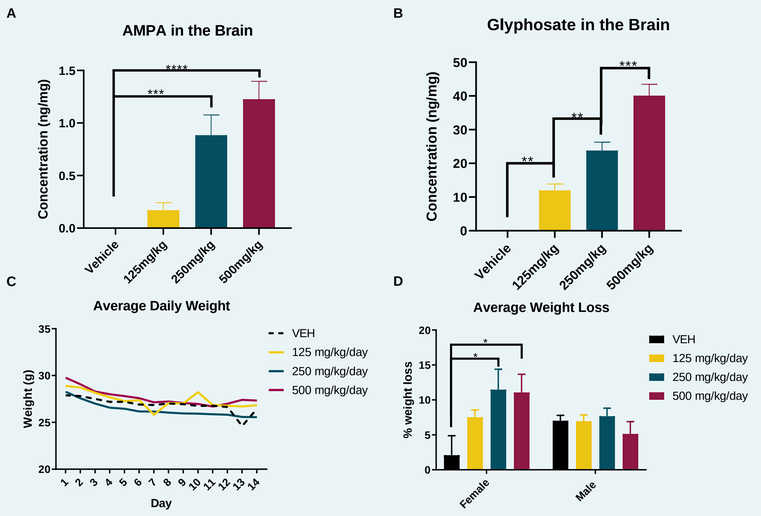


Figure 5. Effect of Glyphosate Treatment on (**A**) concentration of glyphosate found in the brain (ng/mg), (**B**) concentration of Aminomethylphosphonic Acid (AMPA), the major metabolite of glyphosate, found in the brain (ng/mg) (**C**) bodyweight by day (g), and (**D**) average percent weight loss after 2 weeks.

Identify the glyphosate dosages in mice that yield urine glyphosate measurements equivalent to our low and high levels in humans

- Currently, we are determining where the mouse urine glyphosate levels fall in relation to the human urine levels.
- We will then derive a low and a high dosage to imitate low and high levels of human exposure, which we will then use in our future directions.

FUTURE DIRECTIONS

Investigate the effects of glyphosate exposure on cognition and AD pathology in the APP/PS1 mouse model of AD

- We will use the APP/PS1 mouse model of Alzheimer's Disease backcrossed onto a 129/SvJ background (Figure 6).
- Mice will be given dosages of glyphosate determined in the aforementioned study via oral gavage for three months before being introduced to the IntelliCage System for automated behavioral testing (Figure 7, 8) followed by a Rotarod task to assess locomotor function (Table 2; Timeline 2).

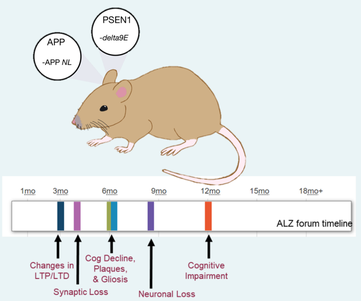
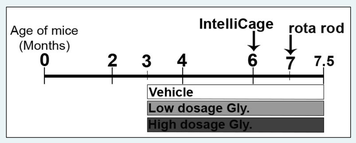


Figure 6. APP/PS1 Mouse Model and onset of AD-related pathology.



Timeline 2. Treatment and behavioral testing. Mice will be tested in the IntelliCage at 6 months of age.

Group	Genotype	Gly. dosage	n
1.	APP/PS1	Vehicle	16
2.	APP/PS1	Low	16
3.	APP/PS1	High	16
4.	NonTg	Vehicle	16
5.	NonTg	Low	16
6.	NonTg	High	16

Table 2. Animal treatment groups and dosages

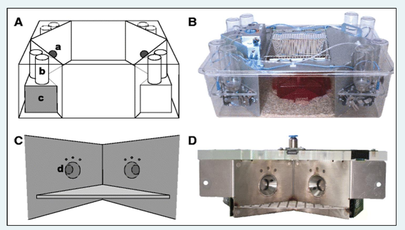


Figure 7. IntelliCage system. The system is composed of one or more cages (**A**, **B**). Through openings (**a**) mice can access bottles (**b**) in a learning chamber (**c**; **C**, **D**). Access to the bottles is controlled by programmable door in smaller openings in the sides of the chamber (**d**). *(Dzik et al., 2018)*

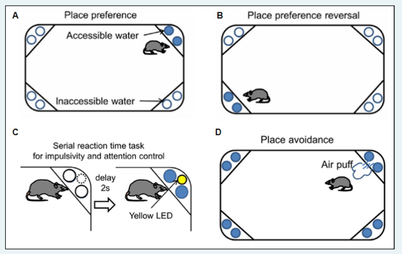


Figure 8. IntelliCage automated behavioral assessment. **A)** Place preference test **B)** Place preference reversal **C)** Serial reaction time task **D)** Place avoidance.

- Following behavioral testing, we aim to elucidate the mechanism through which glyphosate may influence AD pathology through both a biased approach (Figure 9) and through unbiased transcriptomics and metabolomics.
- Molecular analysis will focus on Aβ plaque load, dendritic spine morphology, and APP processing into Aβ plaques (Figure 10).

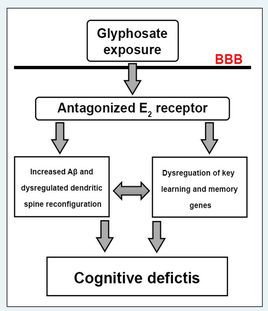


Figure 9. Proposed pathway by which glyphosate induces cognitive deficits.

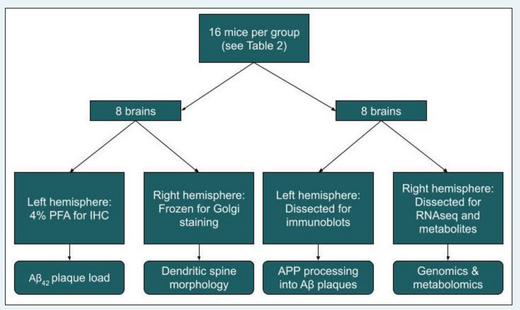


Figure 10. Overview of molecular tissue analysis. *(Abbreviations: PFA-Paraformaldehyde, IHC-immunohistochemistry)*

CONCLUSIONS

- Here we show for the first time in an *in vivo* model that glyphosate crosses the BBB in a dose dependent manner.
- AMPA, the major metabolite of glyphosate, is also found in the brain, but to a lower degree. This may be due to some glyphosate being metabolized by the microbiome.
- Results from future studies will provide valuable insight into glyphosate's potential as a risk factor for AD

Scan me for more info on the Velazquez Lab !