B.N., 2008, Curr. Sci. 94: 459-464; Singh, B.N., S. Singh, and P. Banerjee 2012, Dros. Inf. Serv. 95; Sturtevant, A.H., 1942, Univ. Tex. Publ. 4213: 5–51; Tobari, Y.N., 1993, *Drosophila ananassae* – Genetical and Biological Aspects, Japan Scientific Societies Press, Tokyo; Umina, P.A., *et al.*, 2005, Science 308(5722): 691-693.

Magnesium supplementation reduces seizure and paralysis in *technical knockout* and *easily shocked* bang-sensitive mutants.

Woinski, Alexander, and Elaine R. Reynolds[1]. Biology Department and Neuroscience Program, Lafayette College, 111, Kunkel Hall, Easton, PA 18042; [1]corresponding author: reynolde@lafayette.edu, 610-330-5654.

Introduction

The idea that diet and nutrition play a role in health disorders is not a new one. Nutritional factors have been shown to be involved in the regulation of electrical activity in the brain (Gaby, 2007). In particular, maintenance of proper electrolyte concentrations are important, since their disruption commonly results in seizure and convulsions (Castilla-Guerra *et al.*, 2006). Epilepsy is a common seizure disorder that is treated by anticonvulsants; however, these drugs have limited efficacy in many patients. Because drugs have been somewhat ineffective, other treatments have been sought including diets. For example, the ketogenic diet, which is high in fat and low in carbohydrates and protein, has been utilized in controlling epilepsy in children (Lutas and Yellen, 2013).

Magnesium (Mg) has been used as an anticonvulsant for the condition of pre-eclampsia (Castilla-Guerra *et al.*, 2006). However, the electrolyte has not been used as a treatment for general epilepsy. There are many properties of Mg that may make it a potentially effective anticonvulsant. Mg is the fourth most common mineral found in the human body and is required for over 300 enzyme systems and as a cofactor for mitochondrial energy production. Magnesium also inhibits NMDA receptors, increases prostaglandin synthesis, stabilizes neuronal membranes, and acts as a calcium channel blocker (Castilla-Guerra *et al.*, 2006). Mg deficiency also has been shown to cause seizures or increase susceptibility to seizures. Sufferers of grand mal seizures have been found to maintain lower serum concentrations of magnesium than controls (Gaby, 2007). In a study of hippocampal seizures in rats, injection of $MgSO_4$ proved to increase the rats' seizure thresholds (Hallak *et al.*, 1992). In addition, Mg content in food has been in decline over the past sixty years and up to 75% of Americans do not take in the recommended dietary allowance of the mineral (Yuen and Sander, 2012).

Our study examines if magnesium supplementation could alter seizure behaviors in bang-sensitive mutants that are models of mitochondrial disease and epilepsy. Bang-sensitive (BS) paralytics exhibit seizures and paralysis in response to mechanical stimulation (Reynolds *et al*., 2004). They also show a reduced threshold for seizures compared to wild-type in electrophysiology experiments (Kuebler and Tanouye, 2000). Bang-sensitive mutants include *bangsenseless* (*bss*), *bang-sensitive* (*bas*), *easily shocked* (*eas*), and *technical knockout* (*tko*), among others that express the bang-sensitive phenotype, but with differing biological underpinnings. We found that *eas* and *tko* mutants raised on diets supplemented with Mg would experience decreased seizures and shorter recovery times when exposed to stress conditions.

Methods and Procedures

Flies

Laboratory stock cultures of *tko* and *eas* flies as well as the CS wild-type background were used for the experiments. Flies were cultured at 25°C under 12 hr dark/light conditions on standard molasses/yeast/cornmeal (MYC) food with and without 30 mM $MgSO_4$.

Testing

After eclosion, flies were anesthetized with carbon dioxide and distributed to holding vials for 24 hrs in groups of approximately 10. Flies were then moved to test vials and rested for 15 min. Each vial was vortexed on the highest power setting for ten seconds and was only tested once. The total number of flies in each vial was recorded as well as the number that were paralyzed or seizing on their backs after the ten seconds and the recovery time to standing for each individual fly.

Analysis

A percent paralysis was calculated for each vial and the average percentage was computed for each condition. Average recovery time was assessed for each condition by averaging the recovery times for each individual fly that was paralyzed. Statistical comparisons were made using T tests assuming equal variance (all samples showed equal variance) and using a p value of 0.05 as significant.

Results

The results of the experiments are presented in Table 1. CS flies showed no evidence of seizure or paralysis in either the control or experimental condition. The bang-sensitive mutants *eas* and *tko* both showed a significant improvement in the percentage of flies that seized/paralyzed (91% *vs*. 74% and 95% *vs*. 40%, respectively). The recovery time for both mutants was also reduced by the Mg supplementation. The mutant *tko* was more greatly impacted in terms of percent paralysis, but less impacted in terms of recovery time.

Table 1. The impact of 30 mM magnesium supplementation on bang-sensitive paralysis and recovery.

Strain	Condition	Replicates (N recovery)	% paralysis	T test P value	Recovery in sec	T test P value
CS	No Mg	11	0		--	
	Mg	11	0		--	
eas	No Mg	11 (107)	0.91 ± 0.09	< 0.001	99 ± 40	< 0.0002
	Mg	11 (99)	0.74 ± 0.09		76 ± 41	
tko	No Mg	11 (105)	0.95 ± 0.09	< 0.0001	82 ± 41	0.017
	Mg	11 (51)	0.40 ± 0.11		68 ± 40	

Discussion

Mg supplementation appears to reduce seizure and paralysis in bang-sensitive flies. The amount of Mg increased by supplementation in the food in our experiments is substantial. MYC food is normally about 3 mM for Mg and so the supplemented food is about 10× the normal amount. The results are striking since both mutants were impacted by the supplementation, and to a similar extent observed in feeding antiepileptic drugs such as phenytoin to flies (Reynolds *et al.*, 2004). The gene associated with *eas* encodes ethanolamine kinase, a lipid biosynthetic gene, and the mutant is known to impact lipid composition in the nervous system (Pavlidis *et al*., 1994; Nyako *et al.*, 2001). The gene associated with *tko* encodes a mitochondrial ribosomal protein, and the mutant strain appears to have reduced cytochrome oxidase activity (Royden *et al.*, 1987). Both mutants may interact with Mg through deficits in mitochondrial function or perhaps by stabilizing neuronal membranes. It is possible that Mg deficiency contributes to poor efficacy of anti-epileptic drugs or the prevalence of seizure disorders. While this result is preliminary, it suggests that the interaction between Mg supplementation and epilepsy be further explored.

References: Castilla-Guerra, L., M. Fernandez-Moreno, J.M. Lopez-Chozas, and R. Fernandez-Bolanos 2006, Epilepsia 47: 1990-1998; Gaby, A.R., 2007, Alternative Medicine Review 12: 9-24; Hallak, M., R.F. Berman, S.M. Irtenkauf, M.I. Evans, and D.B. Cotton 1992, American Journal of Obstetrics and Gynecology 167: 1605-1610; Kuebler, D., and M.A. Tanouye 2000, Journal of Neurophysiology 83: 998–1009; Lutas, A., and G. Yellen 2013, Trends in Neurosciences 36: 32-40; Nyako, M., C. Marks, J. Sherma, and E.R. Reynolds 2001, Biochemical Genetics 39: 339 –349; Pavlidis, P., M. Ramaswami, and M.A. Tanouye 1994, Cell 79: 23–33; Reynolds, E.R., E.A. Stauffer, L. Feeney, E. Rojahn, B. Jacobs, and C. McKeever 2004, Journal of Neurobiology 58: 503-513; Royden, C.S., V. Pirrota, and L.Y. Jan 1987, Cell 51: 165–173; Yuen, A.W.C., and J.W. Sander 2012, Epilepsy Research 100: 152-156.

Drosophilid assemblages in burned and unburned vegetation in the Brazilian Savanna.

Leão, B.F.D., R.A. Mata, M.A. Paula, and R. Tidon. Instituto de Biologia, Universidade de Brasília, Brasília, DF, Brasil; Corresponding author: rotidon@pq.cnpq.br

Introduction

The Brazilian Savanna, locally known as Cerrado, is a unique savanna hotspot covering about 2,000,000 km² of South America. It contains savanna vegetation of highly variable structure on the well-drained interfluves, with gallery forests or other wetland vegetation following the watercourses (Oliveira and Marquis, 2002). This biome is highly seasonal, characterized by a distinct dry season from May to September. Wildfire events have been occurring for a long time in this region, as evidenced by the unique array of fire-adapted plant species.

Natural populations of drosophilids have been investigated in this biome, particularly over the past two decades, revealing that the strong environmental heterogeneity impacts the structure of drosophilid assemblages. Communities and populations of these insects decrease through the dry seasons, expand during the rainy seasons, and differ remarkably among vegetation types (Tidon, 2006; Mata *et al.*, 2015). Anthropogenic disturbances, such as urbanization and forests in successional stages, also play a role in drosophilid communities (Ferreira and Tidon, 2005; Mata and Tidon, 2013). Although fire is recognized as a significant form of disturbance due to its potential to influence global ecosystem patterns and processes (Bowman *et al.,* 2009), little is known about the post-fire effects on these flies' assemblages.

In this study, we investigated drosophilid assemblages from forest and savanna patches two years after they had been affected by a severe fire, as well as nearby long-unburned patches used as control sites.

Material and Methods

This research was conducted in the Ecological Reserve of IBGE, located 35 km south of Brasília, the capital of Brazil. This reserve is part of a continuous Environmental Protection Area of 10,000 ha, which has been considered since 1993 by UNESCO to be a core area of the Cerrado Biosphere Reserve. In September 2011, two years before our collections, an accidental and severe fire affected about 90% of the IBGE Reserve and changed its landscape harshly.

Drosophilid samples were carried out bimonthly, from October 2013 to August 2015. On each sampling occasion, four sites representing different habitat types were sampled: (1) burned forest, (2) unburned forest, (3) burned savanna, and (4) unburned savanna. To control for the high heterogeneity of the savanna vegetation, our collections (3 and 4) were made in *cerrado sensu stricto*, a type of savanna very common in the biome. In each site, three sampling units (SU) were established at least 30 m apart. Each SU contained three retention traps (Roque *et al.,* 2011) arranged 10 meters apart. Thus, we came to a standardized sampling effort of three replicates in each one of the four sampled habitats, corresponding to 36 traps per sampling occasion.