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Effects of valproate on seizure-like activity in *Drosophila melanogaster* with a knockdown of *Ube3a* in different neuronal populations as a model of Angelman Syndrome

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ABSTRACT

Angelman Syndrome is a rare, genetically induced neurodevelopmental disorder. This disorder stems from a mutation or deletion of the maternal *UBE3A* gene. Characteristics of this disease include developmental delay, recurring seizures, and severe intellectual disabilities. We studied seizure activity in male *Drosophila melanogaster* with a knockdown of *Ube3a* in different neuronal populations (GABAergic, glutamatergic, mushroom body, and all neurons) and investigated the effects of the antiseizure medication (ASM) on seizure-like activity. Epileptiform activity was monitored in individual fruit flies using imaging chambers and mechanically induced seizures using a vortex assay. A positive control was also used: *eas* (*easily shocked* seizure phenotype). Seizure activity was analyzed for sums of seizure durations, number of seizures, and total time to return to normal activity. *Ube3a* knockdowns in GABAergic neurons elicited more seizure-like episodes than knockdowns in glutamatergic neurons and were on par with the positive control group and those with knockdowns in the mushroom bodies. We have established a method whereby valproate could be administered through food rather than through injections to effectively treat epileptiform activity. We demonstrated that if *Ube3a* is not knocked down pan-neuronally, Angelman Syndrome seizure-like activity can be studied using *Drosophila melanogaster* and therefore allows for high-throughput drug discovery.

Introduction

Angelman Syndrome is a rare, genetically induced neurodevelopmental disorder caused by the absence, or mutation, of the maternal *UBE3A* gene, that codes for E3 ligase [1–5]. This loss of function or apparent absence interferes with protein degradation. Angelman Syndrome is characterized by frequent seizures, severe developmental delay and learning disabilities [1–4]. There is a need for preclinical models that allow for high-throughput drug discovery [6]. *Drosophila melanogaster* is one such preclinical model that has been used previously to study Angelman Syndrome [3,4,7,8] as well as seizure-like activity [9–20]. However, no previous studies have investigated seizure-like activity using *Drosophila melanogaster* as a model organism for Angelman Syndrome in a UAS/GAL4 system.

Previous epilepsy research has used valproate as an antiseizure medication (ASM) and has been shown to assist in the decrease of seizure levels of *Drosophila melanogaster* as well as aid in the prevention of seizures [9–11,21,22]. Valproate increases the concentration of GABA, an inhibitory neurotransmitter in the brain, and inhibits enzymes that are responsible for catabolizing GABA. The results of this drug sum to have profound anti-seizure effects [9,11,21,22]. Since this is the first study to investigate the knockdown of *Ube3a* in a UAS/GAL4 system, we were interested in how this ASM would affect subpopulations of neurons.

In *Drosophila melanogaster*, both wild-type and mutant animals exhibit seizure-like behaviors. However, mutants with seizure phenotypes have greatly reduced stimulus thresholds and the epileptiform episodes have longer durations [13,23–25]. We studied seizure activity in male *Drosophila melanogaster* (fruit flies) with knockdowns of *Ube3a* in different populations of neurons (GABAergic, glutamatergic, mushroom body, and all neurons) both with and without valproate.

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Table 1
Percent seizure-like episodes by genetic line and treatment group.

Genetic Line	Regular Food		Valproate Food	
	# samples exhibiting seizure-like activity/# samples tested	% samples exhibiting seizure-like activity	# samples exhibiting seizure-like activity/# samples tested	% samples exhibiting seizure-like activity
Wild-type	21/36	58.33%	26/49	53.06%
eas	59/73	80.82%	30/54	55.56%
UAS-*Ube3a* (RNAi) × Vglut-GAL4	30/63	47.62%	13/41	31.71%
UAS-*Ube3a* (RNAi) × GAD1-GAL4	47/51	92.16%	40/48	83.33%
UAS-*Ube3a* (RNAi) × Tab2-GAL4	37/43	86.05%	36/50	72.00%
UAS-*Ube3a* (RNAi) × elav-GAL4	6/50	12.00%	12/45	26.67%

Note: The total sample numbers tested and the number that exhibited seizure-like episodes are shown. Fig. 1a includes all *Drosophila* tested while Fig. 1b and Supplemental Fig. 1 only include data from the flies that exhibited seizures for each group, to characterize those seizures.

Materials and methods

Animals

Drosophila melanogaster strains were reared on Nutri-Fly® Bloomington Formulation fly food and studied at room temperature (22–24 °C). Stocks obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study. w^{1118} was used as our wild-type control. The bang-sensitive (BS) phenotype we used as our positive control was the *easily shocked* gene (*eas*) mutant that encodes ethanolamine kinase. For specific neuronal populations we used pan-neuronal elav-GAL4, glutamatergic specific Vglut-GAL4, GABAergic specific GAD1-GAL4 and mushroom body specific Tab2-GAL4. We crossed the GAL4 lines with a transgenic line that expresses dsRNA for RNAi of *Ube3a* under UAS control in the VALIUM10 vector. For additional information regarding fly stocks, please see the Supplemental Materials and Methods.

Drugs

We used 0.5 mM sodium valproate administered in the food. A previous study using *Drosophila melanogaster* as a model organism administered valproate in fly food and found this dose to have a significant effect on circadian rhythms, but lower toxicity compared to higher doses [21]. Other studies investigating seizure-like activity have used higher doses of valproate but administered it through injection prior to electrophysiology studies [9,11].

Mechanical seizure induction

Adult flies were collected 3–4 days posteclosion into their individual imaging chambers. Flies were left to recover for 1–2 h post-CO_2 anesthesia per previously published protocols [15]. Mechanical seizure induction was performed by vortexing the clear plastic imaging chamber (2.7 × 2.7 × 1.3 cm) containing the fly for 10 s at maximum speed. The imaging chambers were then placed under a stereomicroscope, with a camera connected to the device, to record the seizure activity for up to 5 min. A brief vortex mechanical seizure induction leads to uncoordinated hyperactivity or seizure-like activity [12–15]. All video analysis was completed using iMovie.

Statistical analysis

GraphPad Prism 9 was used for all statistical analysis. A two-tailed Grubbs' test with an alpha level of 0.05 was performed to identify any outliers. At most one outlier was removed per group and not all groups contained an outlier. Two-way ANOVAs were used for statistical comparisons between genetic line and treatment group (valproate food vs. regular food). Data are presented as mean ± standard error of the mean (SEM). Asterisks above a bracket denote comparisons between two specific genotypes. Dunnett's multiple comparison tests were used to test for differences of each group compared to the control group (ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$).

Results

Seizure frequency across test groups

The wild-type line and the UAS-*Ube3a* (RNAi) × Vglut-GAL4 lines had the least % population that exhibited seizures among the genetic lines (Table 1). In addition, they also had the least # of seizures (Fig. 1a). Valproate lessened both the % of samples that exhibited seizures (Table 1) and the average number of seizures exhibited per testing session (Fig. 1a). These data include all flies that were tested. The two-way ANOVA of the # of seizures revealed not only a significant difference in genetic lines ($F = 51.74$, $p < 0.0001$), but there was also a statistically significant difference between regular food vs. valproate food ($F = 35.14$, $p < 0.0001$), as well as an interaction effect ($F = 8.011$, $p < 0.0001$) (Fig. 1a).

Characterization of epileptiform activity

Previous studies have shown that flies follow a specific pattern of 6 seizure phases including initial seizure, paralysis, tonic-clonic phase, recovery seizure, refractory period, and recovery [24,26,27]. Many studies include data only on the time it takes for the fly to resume normal activity. We found a lot of variability in the number of epileptiform episodes that occurred for each fly and wanted to further characterize these seizure-like episodes. Therefore, we evaluated the # of seizures, the duration of each, and evaluated the sum of all seizure durations per testing session. We defined the total elapsed time until the flies resumed normal activity as the last timepoint of the final seizure to reduce subjectivity of "normal activity" which also varied among genetic lines and treatment groups tested. We were also very interested in characterizing the seizures themselves. For this reason, while measuring the total elapsed time to return to normal activity and the sum of all seizure durations, we excluded flies that did not have seizures as to not skew the data. If this data were to be included, there would have most likely been more statistically significant differences among the groups tested.

There was no statistical difference among total seizure durations in flies that exhibited seizures compared to the control group (wild-type flies in regular food; Supplemental Fig. 1). However, the two-way ANOVA for the time to return to regular activity did reveal significant differences in the genetic lines ($F = 6.806$, $p < 0.0001$) between the treatment groups of regular food vs. valproate ($F = 12.08$, $p = 0.0006$). There was also an interaction effect which was statistically significant ($F = 3.054$, $p = 0.0103$). Lastly, the Dunnett's multiple comparisons test also found statistical differences in the UAS-*Ube3a* (RNAi) × Glut-GAL4

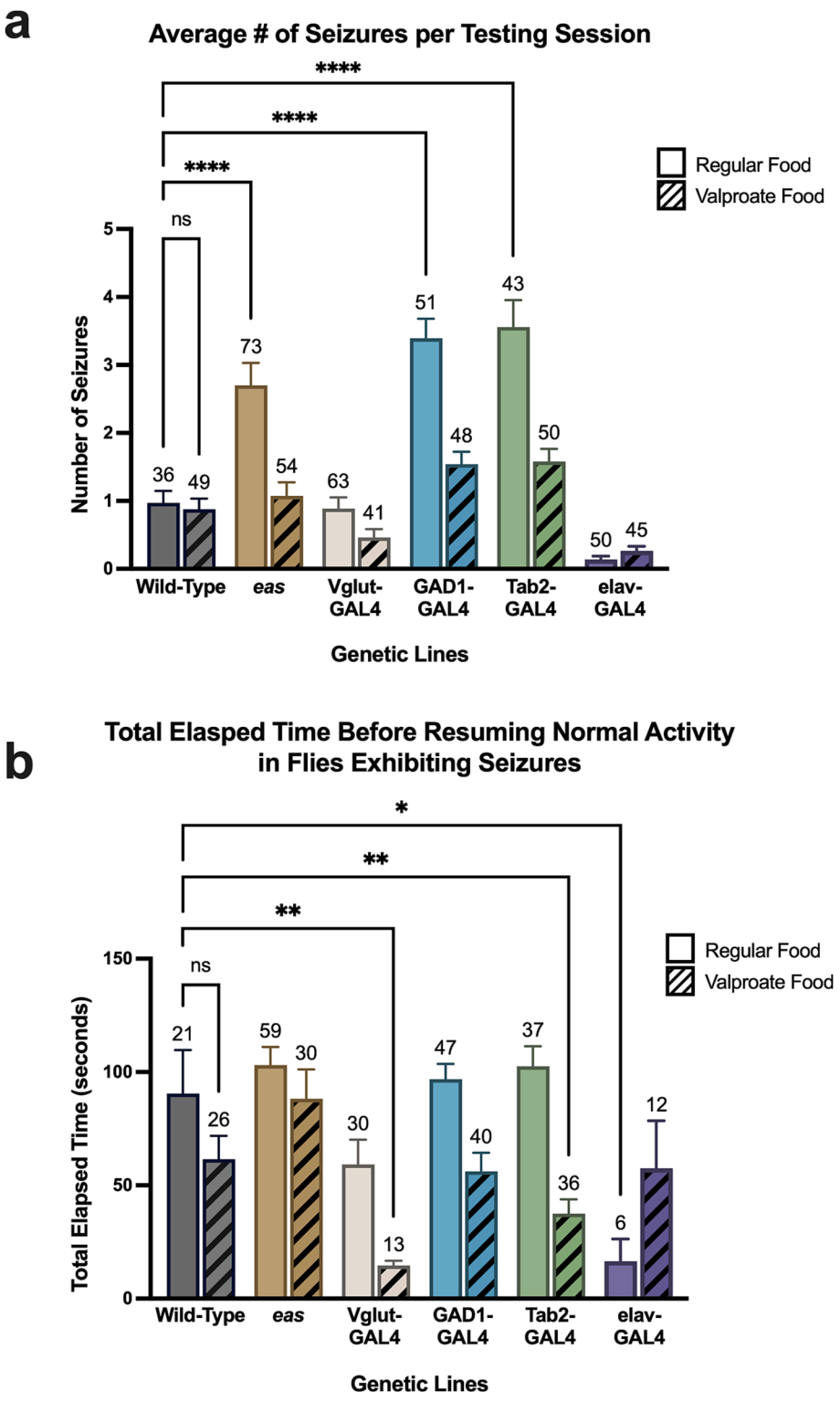

Fig. 1. Characterization of seizure-like activity. Each of the GAL4 lines were crossed with UAS-*Ube3a* (RNAi) lines and the male progeny were the test subjects shown above. a) Average number of seizure-like episodes per testing session of all male flies studied are shown by genetic lines of flies and includes both flies raised in regular food (solid bars) vs. food with valproate (bars with diagonal lines). b) Total elapsed times before resuming normal activity are shown for male flies that exhibited seizure-like episodes; excludes flies that didn't have seizure-like episodes. Includes both male flies raised in regular food (solid bars) vs. food with valproate (bars with diagonal lines). Means are shown +/- SEM. A Dunnett's multiple comparison test was used to test for differences of each group compared to the control group (ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ****: $p < 0.0001$). Sample numbers are indicated above each bar.

and UAS-*Ube3a* (RNAi) × Tab2-GAL4 lines in the valproate treatment groups compared to the control group (wild-type on regular food) (Fig. 1b).

Discussion

Three of the groups, including the positive control — *eas* flies that exhibit a seizure phenotype, *Ube3a* (RNAi) flies with knockdowns in GABAergic neurons (GAD-GAL4) and mushroom bodies (Tab2-GAL4), all had more than 80% of the samples tested that exhibited seizure-like episodes (Table 1). This demonstrates that the *Ube3a* knockdown affects GABAergic neurons more that glutamatergic neurons in a way that elicits more seizure-like episodes. This data is consistent with previous studies that showed the UAS-*eas* + phenotype expressed in GAD-GAL4 were similar to the *eas* flies in electrophysiology studies using heat-shock to induce seizure-like episodes [16]. Although Angelman Syndrome is caused by mutation or deletion of the maternal *UBE3A* gene, a region that is commonly deleted in Angelman Syndrome contains genes that code for different subunits of $GABA_A$ receptors [28]. In addition, a previous study in mice has shown GABAergic synapse deficits with a loss of maternal *Ube3a* [29]. Possible future therapeutics have been summarized by Samanta (2020) and include viral vector therapy [28]. Taken with our current study, this therapy would seem to be most effective by targeting GABAergic neurons specifically.

Valproate treatment reduced the percent population that exhibited seizures across all groups (Table 1). This demonstrates that administering valproate to *Drosophila melanogaster* in their food is a viable research method for future studies rather than through injections of higher doses of valproate. Although valproate as an ASM is no longer the first-line treatment for individuals with Angelman Syndrome due to side effects [28], it has been used often in previous studies using *Drosophila melanogaster* and can be used in future studies to compare newer treatments in the control of epileptiform activity.

Different bang-sensitive mutants exhibit a variety of refractory periods during seizure-like episodes (2 min to an hour has been previously observed) [13,14]. The *eas* flies were found to have shorter refractory periods [14]. Our data was in agreement with these earlier findings, as multiple seizure-like episodes occurred in the *eas* flies. The analysis of the sum of seizures durations was not found to be statistically significant (Supplemental Fig. 1).

Interestingly, one previous study showed that *Ube3a* mutants did not exhibit seizure-like episodes, however, this study was done in mutants that had *Ube3a* knocked down globally [4]. Other previous studies have shown that overexpression, rather than knockdown, of *Ube3a* can also lead to seizures and learning disabilities in *Drosophila melanogaster* as a model of Autism. When there was an overexpression of *Ube3a* in all neurons by elav-GAL4 there was no bang-sensitive phenotype [20]. In the same study, glia-specific overexpression of *Ube3a* exerted resulted in seizures induced through bang sensitivity. Takei et al. suggested that the ubiquitin ligase activity of *Ube3a* is essential for bang sensitivity [26]. Our results with pan-neuronally expressed *Ube3a* (RNAi) through the elav-GAL4 mutant is in line with both studies, as they had the lowest % population that exhibited seizures. In addition, we raised the flies at room temperature and RNAi knockdown using the GAL4 system has been shown to be more effective at higher temperatures [30–33]. This research also demonstrates that if *Ube3a* is not knocked down pan-neuronally, Angelman Syndrome seizure-like activity can be studied using *Drosophila melanogaster* and therefore allows for high-throughput drug discovery. Use of this model organism affords faster lifecycles and allows for more cost-efficient and timely initial drug screening studies prior to testing novel therapies in higher-order organisms. Future studies investigating neuronal architecture and possible microcephaly would greatly enhance literature in this area. Following identification of possible therapeutics using this model organism, future studies could be conducted on zebrafish [6] and mice [34].

Conclusions

In accordance with previously published data, valproate was shown to be an effective treatment for reducing the number of seizure-like episodes for flies exhibiting Angelman Syndrome. We have established a method whereby valproate could be administered through food rather than through injections to effectively treat epileptiform activity. When there was an under expression of *Ube3a* in all neurons through RNAi by elav-GAL4 there was a significant reduction in bang-sensitivity. Flies with the *Ube3a* knockdown in GABAergic neurons were significantly more effected than knockdown in glutamatergic neurons in a way that elicited more seizure-like episodes. We demonstrated that if *Ube3a* is not knocked down pan-neuronally, Angelman Syndrome seizure-like activity can be studied using *Drosophila melanogaster* and therefore allows for high-throughput drug discovery.

Limitations

Although there were significant differences between the groups and controls were used, the number of seizures in the wild-type group was still high. Seizure induction methods vary widely [15]. It should be noted that there is a large amount of variability in the literature even among mechanical seizure induction methods in *Drosophila melanogaster* [15,17–19,25]. We feel that either this is likely the cause of this variability, along with vortexing of the subjects in an imaging chamber with a different geometry than the standard empty fly vial. Future studies should be conducted to investigate and help standardize mechanical seizure induction protocols.

Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Both authors approved the final version of the manuscript to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Both authors have agreed to the submission.

CRediT authorship contribution statement

Madeline C. Moore: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **Danielle T. Taylor:** Investigation, Validation, Formal analysis, Supervision, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendices A and B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebr.2023.100622.

References

[1] Dagli A, Buiting K, Williams CA. Molecular and clinical aspects of angelman syndrome. Mol Syndromol 2012;2(3-5):100–12. https://doi.org/10.1159/000328837.

[2] Clayton-Smith J. Angelman syndrome: a review of the clinical and genetic aspects. Journal of Medical Genetics 2003;40:87–95. https://doi.org/10.1136/jmg.40.2.87.

[3] Li W, Yao A, Zhi H, Kaur K, Zhu Y-C, Jia M, et al. Angelman syndrome protein Ube3a regulates synaptic growth and endocytosis by inhibiting BMP signaling in drosophila. PLoS Genetics 2016;12(5):e1006062. https://doi.org/10.1371/journal.pgen.1006062.

[4] Wu Y, Bolduc FV, Bell K, Tully T, Fang Y, Sehgal A, et al. A *Drosophila* model for Angelman syndrome. Proceedings of the National Academy of Sciences 2008;105(34):12399–404. https://doi.org/10.1073/pnas.0805291105.

[5] Reiter LT, Seagroves TN, Bowers M, Bier E. Expression of the Rho-GEF Pbl/ECT2 is regulated by the UBE3A E3 ubiquitin ligase. Human Molecular Genetics 2006;15:2825–35. https://doi.org/10.1093/hmg/ddl225.

[6] Shcheglovitov A, Peterson RT. Screening platforms for genetic epilepsies—Zebrafish, iPSC-Derived neurons, and organoids. Neurotherapeutics 2021;18:1478–89. https://doi.org/10.1007/s13311-021-01115-5.

[7] Lu Y, Wang F, Li Y, Ferris J, Lee J-A, Gao F-B. the drosophila homologue of the angelman syndrome ubiquitin ligase regulates the formation of terminal dendritic branches. Human Molecular Genetics 2008;18:454–62. https://doi.org/10.1093/hmg/ddn373.

[8] Gatto CL, Broadie K. Drosophila modeling of heritable neurodevelopmental disorders. Current Opinion in Neurobiology 2011;21:834–41. https://doi.org/10.1016/j.conb.2011.04.009.

[9] Kuebler D, Tanouye M. Anticonvulsant valproate reduces seizure-susceptibility in mutant drosophila. Brain Research 2002;958:36–42. https://doi.org/10.1016/S0006-8993(02)03431-5.

[10] Song J, Tanouye M. From bench to drug: Human seizure modeling using drosophila. Progress in Neurobiology 2008;84:182–91. https://doi.org/10.1016/j.pneurobio.2007.10.006.

[11] Howlett IC, Tanouye MA. Seizure-Sensitivity in *Drosophila* Is Ameliorated by Dorsal Vessel Injection of the Antiepileptic Drug Valproate. Journal of Neurogenetics 2013;27:143–50. https://doi.org/10.3109/01677063.2013.817574.

[12] Song J, Parker L, Hormozi L, Tanouye MA. DNA topoisomerase I inhibitors ameliorate seizure-like behaviors and paralysis in a drosophila model of epilepsy. Neuroscience 2008;156:722–8. https://doi.org/10.1016/j.neuroscience.2008.07.024.

[13] Pavlidis P, Ramaswami M, Tanouye MA. The drosophila easily shocked gene: A mutation in a phospholipid synthetic pathway causes seizure, neuronal failure, and paralysis. Cell 1994;79:23–33. https://doi.org/10.1016/0092-8674(94)90397-2.

[14] Pavlidis P, Tanouye M. Seizures and failures in the giant fiber pathway of drosophila bang- sensitive paralytic mutants. The Journal of Neuroscience 1995;15:5810–9. https://doi.org/10.1523/JNEUROSCI.15-08-05810.1995.

[15] Mituzaite J, Petersen R, Claridge-Chang A, Baines RA. Characterization of Seizure Induction Methods in *Drosophila*. Eneuro 2021;8:ENEURO.0079-21.2021. https://doi.org/10.1523/ENEURO.0079-21.2021.

[16] Kroll JR, Tanouye MA. Rescue of *Easily shocked* mutant seizure sensitivity in *Drosophila* adults: Ethanolamine Kinase and Seizure Susceptibility. Journal of Comparative Neurology 2013;521:3500–7. https://doi.org/10.1002/cne.23364.

[17] Tan JS, Lin F, Tanouye MA. Potassium bromide, an anticonvulsant, is effective at alleviating seizures in the drosophila bang-sensitive mutant bang senseless. Brain Research 2004;1020:45–52. https://doi.org/10.1016/j.brainres.2004.05.111.

[18] Stone B, Evans L, Coleman J, Kuebler D. Genetic and pharmacological manipulations that alter metabolism suppress seizure-like activity in drosophila. Brain Research 2013;1496:94–103. https://doi.org/10.1016/j.brainres.2012.12.007.

[19] Stone B, Burke B, Pathakamuri J, Coleman J, Kuebler D. A low-cost method for analyzing seizure-like activity and movement in drosophila. Journal of Visualized Experiments 2014:51460. https://doi.org/10.3791/51460.

[20] Hope KA, LeDoux MS, Reiter LT. Glial overexpression of Dube3a causes seizures and synaptic impairments in drosophila concomitant with down regulation of the Na+/K+ pump ATPα. Neurobiology of Disease 2017;108:238–48. https://doi.org/10.1016/j.nbd.2017.09.003.

[21] Dokucu ME, Yu L, Taghert PH. Lithium- and Valproate-Induced alterations in circadian locomotor behavior in drosophila. Neuropsychopharmacology 2005;30:2216–24. https://doi.org/10.1038/sj.npp.1300764.

[22] Cunliffe VT, Baines RA, Giachello CNG, Lin W-H, Morgan A, Reuber M, et al. Epilepsy research methods update: Understanding the causes of epileptic seizures and identifying new treatments using non-mammalian model organisms. Seizure 2015;24:44–51. https://doi.org/10.1016/j.seizure.2014.09.018.

[23] Ganetzky B, Wu C-F. indirect suppression involving behavioral mutants with altered nerve excitability in drosophila melanogaster. Genetics 1982;100:597–614. https://doi.org/10.1093/genetics/100.4.597.

[24] Parker L, Howlett IC, Rusan ZM, Tanouye MA. Seizure and Epilepsy: Studies of Seizure Disorders in Drosophila. Int. Rev. Neurobiol., vol. 99, Elsevier; 2011, p. 1–21. https://doi.org/10.1016/B978-0-12-387003-2.00001-X.

[25] Kuebler D, Tanouye MA. Modifications of seizure susceptibility in *Drosophila*. Journal of Neurophysiology 2000;83:998–1009. https://doi.org/10.1152/jn.2000.83.2.998.

[26] Takai A, Yamaguchi M, Yoshida H, Chiyonobu T. Investigating developmental and epileptic encephalopathy using drosophila melanogaster. International Journal of Molecular Sciences 2020;21:6442. https://doi.org/10.3390/ijms21176442.

[27] Parker L, Padilla M, Du Y, Dong K, Tanouye MA. Drosophila as a Model for Epilepsy: *bss* Is a Gain-of-Function Mutation in the Para Sodium Channel Gene That Leads to Seizures. Genetics 2011;187:523–34. https://doi.org/10.1534/genetics.110.123299.

[28] Samanta D. Epilepsy in angelman syndrome: A scoping review. Brain Dev 2021;43:32–44. https://doi.org/10.1016/j.braindev.2020.08.014.

[29] Wallace ML, Burette AC, Weinberg RJ, Philpot BD. Maternal loss of Ube3a produces an excitatory/Inhibitory imbalance through neuron Type-Specific synaptic defects. Neuron 2012;74:793–800. https://doi.org/10.1016/j.neuron.2012.03.036.

[30] Fortier E, Belote JM. Temperature-dependent gene silencing by an expressed inverted repeat in drosophila. genes N Y N 2000;2000(26):240–4. https://doi.org/10.1002/(sici)1526-968x(200004)26:4<240::aid-gene40>3.0.co;2-p.

[31] Ni J-Q, Markstein M, Binari R, Pfeiffer B, Liu L-P, Villalta C, et al. Vector and parameters for targeted transgenic RNA interference in drosophila melanogaster. Nature Methods 2008;5(1):49–51. https://doi.org/10.1038/nmeth1146.

[32] Ni J-Q, Liu L-P, Binari R, Hardy R, Shim H-S, Cavallaro A, et al. A Drosophila Resource of Transgenic RNAi Lines for Neurogenetics. Genetics 2009;182:1089–100. https://doi.org/10.1534/genetics.109.103630.

[33] Kaya-Çopur A, Schnorrer F. A guide to Genome-Wide in vivo RNAi applications in drosophila. Methods Mol Biol Clifton NJ 2016;1478:117–43. https://doi.org/10.1007/978-1-4939-6371-3_6.

[34] Syding LA, Kubik-Zahorodna A, Nickl P, Novosadova V, Kopkanova J, Kasparek P, et al. Generation and characterization of a novel angelman syndrome mouse model with a full deletion of the Ube3a gene. Cells 2022;11(18):2815. https://doi.org/10.3390/cells11182815.