Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 01, pp.25075-25079, January, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

CLIMBING RESPONSES OF FEW SPECIES OF *DROSOPHILA* ON EXPOSURE TO DIFFERENT ANITI EPILEPTIC DRUGS

***Harini, B. P.**

Drosophila Culture Laboratory, Department of Zoology, Bangalore University, Bangalore- 560 056, Karnataka, India

ARTICLE INFO

Article History:
Received 22nd October, 2015
Received in revised form
27th November, 2015
Accepted 15th December, 2015
Published online 31st January, 2016

Key words:

Drosophila, Anti epileptic drugs, Climbing ability, Geo taxis.

ABSTRACT

Feeding flies on wheat cream agar media supplemented with antiepileptic drugs are studied for adult locomotary behaviors like preferential adult geotaxis. The behavioral plasticity of climbing ability of an adult is an important event with decisive influence on their preference and capability. *Drosophila* sensory systems contribute to detect, localize and provide information about the availability of food and chemical features of environments. The present study revealed that the Dose dependent action of different AEDs produced a maximal effect on behaviors of *Drosophila* species and provides an efficient system to study genetic, neurological, and behavioral mechanisms mediating these effects. Irrespective of the species the responses are similar on exposure to higher doses with decreased activity. AED has an important role in regulating behavior through metabolism; such studies should be useful for understanding the multiple effects on behavior and health.

Citation: **Harini, B.P. 2016.** "Climbing responses of few species of *drosophila* on exposure to different Aniti epileptic drugs", *International Journal of Current Research,* 8, (01), 25075-25079.

INTRODUCTION

Antiepileptic drugs (AEDs) have a variety of mechanisms of action which are reflected through different anticonvulsant activities and behavioral effects (Cavanna *et al*., 2010). Several human studies have raised concerns over AED behavioral teratogenesis. Animal studies have demonstrated that AEDs can produce cognitive deficits at dosages less than those required for anatomical teratogenesis (Fisher and Vorhees, 1992). Anatomical and behavioral teratogenesis likely differ in mechanisms since first trimester AED exposure poses the highest risk for anatomical malformations, while third trimester exposure appears to be associated with the highest risk for adverse behavioral effects (Gaily and Meador, 2007). Studies in rats have shown significant AED effects in the developing brain including apoptotic neurodegeneration (Olney *et al*., 2002; Bittigau *et al*., 2003);

****Corresponding author: Harini, B.P.,***
Drosophila Culture Laboratory, Department of Zoology, Bangalore University, Bangalore- 560 056, Karnataka, India.

neurodevelopmental delay, behavioral disorders or learning disabilities as an outcome of in utero exposure to AEDs and specially VPA (Nicolai *et al*., 2008). The cognitive side effects of CBZ, PHT and VPA are comparable and associated with modest psychomotor slowing accompanied by decreased attention and memory (Meador, 2005). PHT is implicated in dose related decline in concentration, memory and mental speed, as well as generating anxiety, aggression, fatigue, and depression (Gillham *et al*., 1990). Sedation and outbursts of psychotic episodes have been described with PHT at high doses (Levinson and Devinsky, 1999). PHT produces multiple behavioral dysfunctions in rat offspring at sub teratogenic and non growth retarding doses (Adams *et al*., 1990).

The chronic use of VPA can impair concentration, and also reversible Parkinsonism and cognitive impairment (Nicolai *et al*., 2008). There is a better recognition of the behavioral phenotype in Fetal valproate syndrome (Williams *et al*., 2001; Dean *et al*., 2002). Poor concentration and hyperactivity have also been commonly reported on VPA exposure (Kini, 2006). The active metabolite carbamazepine epoxide is partly responsible for the mild cognitive and psychomotor effects attributed to CBZ (Gillham *et al*., 1988).

The exposure of pregnant rats to CBZ significantly delayed skull bone development and soft tissues flattening, these structural alterations brought confrontational changes associated to the behavior parameters of the offspring (Christensen *et al*., 2004; Rayburn *et al*., 2004). A recent CNS practice guideline stated, "Behavioral and cognitive side effects need to be better evaluated and individual risks as well as group differences assessed on tests of cognition" (Hirtz *et al*., 2003). Behavioral side effect profiles of AEDs, both negative and positive psychotropic effects, should be considered in the choice of the optimal drug for an individual patient (Schmitz, 2006).

At the forefront of behavioral genetics research, D. melanogaster has provided important insights into the molecular, cellular and evolutionary basis of behavior (Sokolowski, 2001). Simple behavioral assays are widely applicable for studying the role of genetic and environmental factors on fly behavior on exposure to few AEDs (Sharma *et al*., 2010). The newly hatched adult fly will rapidly acquire characteristic behaviors of flight, chemotaxis, phototaxis, geotaxis, foraging and mating (Truman *et al*., 1993). In many cases the explicit circuits controlling visual (Ting and Lee, 2007), olfactory (Hallem and Carlson, 2004), mechanosensory (Kernan, 2007) and chemosensory (Stocker, 1994) inputs from the peripheral organs (eye, antennae, bristle organs and maxillary palps) have been mapped both physically and functionally. In addition, the central 'mushroom body' of the brain has been elucidated as a center for memory and conditioned behaviors.

Some of these well documented developmental and behavioral aspects of Drosophila make it an especially informative and adaptable model to investigate a wide variety of toxicological endpoints relevant to human biology and behavior. Flies exhibit a wide array of behaviors relevant to understanding human response to environmental challenges. These behaviors include locomotion, circadian rhythm, sleep patterns, courtship and mating, aggression, and grooming. Many of these are under the control of genetic and molecular mechanisms in Drosophila (Sokolowski, 2001; Greenspan and Dierick, 2004). Furthermore, at a physiological level the underlying neurotransmitter systems in the fly are conserved including serotonin, dopamine, GABA, glutamate and acetylcholine (Nichols, 2006).

To date, behavioral endpoints in Drosophila have been used primarily to isolate genes that specifically support a given trait rather than as a tool for screening vast numbers of chemicals (Moore *et al*., 1998). Locomotor activity is a complex behavior and different neural systems may influence in fly (Fleming and Copp, 1998). It can be assessed in transgenic and mutant flies through longevity assays, locomotor and climbing assays. Progressive locomotor decline can be observed in transgenic Drosophila through climbing assays (Greene *et al*., 2003). The duration of climbing is determined by Rapid Iterative Negative Geotaxis (RING) (startle-induced vertical climbing) as an assay for evaluating the sedative effects of AEDs (Sharma *et al*., 2010). A climbing or negative geotaxis assay measuring the ability of the organisms to climb up the walls of a plastic vial was used and several genotypes or drug treatments can be tested for screening experiments (Nichols *et al*., 2012).

Climbing ability is a frequently used assay to measure locomotor activity in D. melanogaster model of Parkinson's disease (Todd and Staveley, 2004). The climbing response of wild-type flies remained essentially unchanged as reported by Feany and Bender (2000). The climbing response for subsequent anti-parkinson drug studies has an effective in decreasing fly locomotor function (Pendleton *et al*., 2000). The low levels of accumulated ethanol stimulate locomotion and high levels depress it (Heberlein *et al*., 2004). These behavioral assays are widely applicable for studying the role of genetic and environmental factors on fly behavior. In light of the above studies the present work determine the acute behavioral responses to commonly used conventional AEDs with respect to adult geotaxis in Drosophila species at different doses. The present study has been assessed for the dose response relationship between AEDs and their behavior in different species of Drosophila.

MATERIALS AND METHODS

The fly stocks, D. melanogaster, D. ananassae and D. nasuta nasuta were cultured on standard wheat cream agar medium in uncrowded culture condition at 22±1ºC (rearing temperature) with a relative humidity of 70%. The progeny from these stabilized stocks treated with PHT (5, 10 and 15 mg/ml), VPA (0.2, 0.3 and 0.4 mg/ml) and CBZ (2, 4 and 8 mg/ml) were used to assess the larval pupation site preference and climbing ability (negative geotaxis) and compared to respective controls.

Climbing ability

Virgin females and unmated males of D. melanogaster, D. ananassae and D. nasuta nasuta were isolated, collected and aged for 5 days. All flies used in individual experiments were grown, collected and handled in parallel. Adult flies (5 days old) were aspirated, transferred to fresh food vials containing different doses of each antiepileptic drug and treated for 3 days. 20 flies were selected from treated and placed in a 100 ml glass graduated cylinder (length 25 cm and diameter 3 cm) to climb. The cylinder was sealed with parafilm at the top to prevent escape. The flies were gently knocked to the bottom of the cylinder and were allowed to climb for 30 sec. The number of flies crossing the 60 ml line (9 cm) was recorded. Four such trials were conducted for each dose of PHT, VPA and CBZ. The locomotor activity of Drosophila without drug administration i.e., control was compared to treated (modified protocol of Greene *et al*., 2003). The number

of flies climbed in the given time for each dose was averaged for statistical analysis.

RESULTS

Climbing assay performed on adults of Drosophila species exposed to different doses of PHT, CBZ and VPA is presented in Fig 1. The response to increase dose has resulted with decrease climbing activity of flies for all the three variable drugs of PHT, CBZ and VPA. The percentage of flies exhibiting about 50% negative geotaxis was observed at mid and high doses in all the species. At 10 and 15 mg/ml, the climbing responses were 56.25% and 55% in D. melanogaster; 57.5% and 47.5% in D. ananassae; 51.25 and 45% in D. nasuta nasuta respectively with respect to PHT.

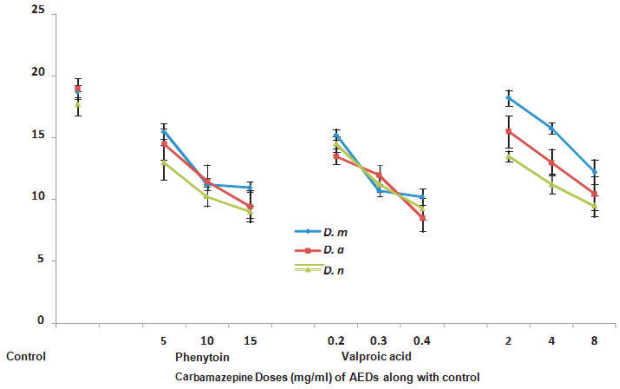

Figure 1. Mean climbing ability of Drosophila species on exposure to different doses

The percentage of flies exhibiting about 50% negative geotaxis was observed at high doses in D. ananassae and D. nasuta nasuta in addition to mid dose in D. nasuta nasuta on exposure to CBZ. At 8 mg/ml the climbing responses were 52.5% and 47.5% in D. ananassae and D. nasuta nasuta while it was 56.25% at 4 mg/ml in D. nasuta nasuta. While on exposure to different doses of VPA the percentage of flies exhibiting about 50% negative geotaxis was observed at mid and high doses in all the species except at 0.3 mg/ml in D. ananassae. At 0.3 and 0.4 mg/ml, the climbing responses were 53.75% and 51.25% in D. melanogaster; 56.25 and 46.25% in D. nasuta nasuta respectively while in D. ananassae (42.5%) was recorded at 0.4 mg/ml.

DISSCUSION

The genetic analysis remains the best means to define mechanisms and to begin the process of assigning the contribution of genes to behavior. The Drosophila flies were exposed to varying doses of antiepileptic drug for three days to determine its effect on behaviors. In preclinical studies on animals, AEDs produce acute adverse effects such as sedation, ataxia, tremor, impairment of motor coordination, disturbance in locomotor activity and alterations in skeletal muscular strength. Grip strength test is able to evaluate the acute adverse effect potential of AEDs at high (neurotoxic) doses with respect to the reduction of muscular strength (Zadroniak *et al*., 2009).

The animals exposed to PHT showed significant increase in locomotor activity measures. These results confirm a small but growing body of literature that demonstrates that PHT is a behavioral teratogen (Pizzi and Jersey, 1992). The observed mean values of locomotor activity were dose dependent and significantly different among different AEDs exposure on Drosophila species. Climbing activity was decreased at mid and high doses with less than 50% of the flies climbing within 30 sec while 95% of the flies climbing within 15 sec in control. Climbing rate of D. nasuta nasuta was reduced on exposure to all the three AEDs compared to D. melanogaster and D. ananassae. Interestingly, the behavioral traits observed were generally dose dependent. The nervous system, the most crucial system in the elicitation of behavior, is formed during development by networks of interacting genes and the physiological structures necessary to generate these behavior patterns. Despite the sources of complexity, the amount of research accomplished has pushed the fruit fly to the forefront of behavioral genetics research (Sokolowski, 2001).

Dose dependent action of different AEDs produced a maximal effect on behaviors of Drosophila species and provides an efficient system to study genetic, neurological, and behavioral mechanisms mediating these effects. AED has an important role in regulating behavior through metabolism; such studies should be useful for understanding the multiple effects on behavior and health.

Acknowledgment

B.P.H. is grateful to Department of Zoology, Bangalore University, Jnanabharathi Campus, Bangalore, for the support and encouragement.

REFERENCES

Adams, J., Vorhees, C.V. and Middaugh, L.D. 1990. Developmental neurotoxicity of anticonvulsants: human and animal evidence on phenytoin. *Neurotoxicol Teratol*., 12: 203-214.

Bittigau, P., Sifringer, M. and Ikonomidou, C. 2003. Antiepileptic drugs and apoptosis in the developing brain. *Ann N Y Acad Sci*., 993: 103-114.

Cavanna, A.E., Ali, F., Rickards, H.E. and McCorry, D. 2010. Behavioral and cognitive effects of anti-epileptic drugs. *Discov Med*., 9: 138-144.

Christensen, H.D., Rayburn, W.F., Parker, K.M., Gonzalez, C.L. and Gold, K.P. 2004. Chronic prenatal exposure to carbamazepine and perinatal outcomes of C3H/He mice. *Am. J. Obstet Gynecol,* 190: 259-263.

Dean, J.C., Hailey, H., Moore, S.J., Lloyd, D.J., Turnpenny, P.D. and Little, J. 2002. Long term health

and neurodevelopment in children exposed to antiepileptic drugs before birth. *J. Med. Genet.,* 39: 251-259.

Feany, M.B. and Bender, W.W. 2000. A Drosophila model of Parkinson's disease. *Nature,* 404:394-398.

Fisher, J.E. and Vorhees, C.V. 1992. Developmental toxicity of antiepileptic drugs: relationship to postnatal dysfunction. *Pharmacol Res.,* 26: 207-221.

Fleming, A. and Copp, A.J. 1998. Embryonic folate metabolism and mouse neural tube defects. *Science*, 280: 2107-2109.

Gaily, E. and Meador, K.J. 2007. Neurodevelopmental effects. In: Epilepsy: A comprehensive textbook. (Eds. Engel, J and Pedley, TA), 2nd Edition. Lippincott Wiliams and Wilkins, Philadelphia. vol II, Section V. pp. 1225–1233.

Gargano, J.W., Martin, I., Bhandari, P. and Grotewiel, M.S. 2005. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in Drosophila. *Exp Gerontol*., 40: 386-395.

Gillham, R.A., Williams, N., Wiedmann, K.D., Butler, E., Larkin, J.G. and Brodie, M.J. 1988. Concentration-effect relationships with carbamazepine and its epoxide on psychomotor and cognitive function in epileptic patients. *J Neurol Neurosurg Psychiatry*, 51: 929-933.

Gillham, R.A., Williams, N., Wiedmann, K.D., Butler, E., Larkin, J.G. and Brodie, M.J. 1990. Cognitive function in adult epileptic patients established on anticonvulsant monotherapy. *Epilepsy Res*., 7: 219-225.

Greene, J.C., Whitworth, A.J., Kuo, I., Andrews, L.A., Feany, M.B. and Pallanck, L.J. 2003. Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc Natl Acad Sci U S A*, 100: 4078-4083.

Greenspan, R.J. and Dierick, H.A. 2004. 'Am not I a fly like thee?' From genes in fruit flies to behavior in humans. Hum Mol Genet 13 Spec No 2, R267-273.

Hallem, E.A. and Carlson, J.R. 2004. The odor coding system of Drosophila. Trends Genet 20: 453-459.

Heberlein, U., Wolf, F.W., Rothenfluh, A. and Guarnieri, D.J. 2004 Molecular genetic analysis of ethanol intoxication in Drosophila melanogaster. *Integr Comp Biol*., 44: 269-27.

Hirtz, D., Berg, A. and Bettis, D. 2003. Practice parameter: treatment of the child with a first unprovoked seizure: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology,* 60: 166-175.

Kernan, M.J. 2007. Mechanotransduction and auditory transduction in Drosophila. *Pflugers Arch*., 454: 703-720.

Kini, U. 2006. Fetal valproate syndrome: a review. *Paediatr Perinat Drug Ther*., 7:123- 130.

Levinson, D.F. and Devinsky, O. 1999. Psychiatric adverse events during vigabatrin therapy. *Neurology,* 53, 1503-1511.

Meador, K.J. 2005. Cognitive effects of epilepsy and anti epileptic medications. In: The treatment of epilepsy. Principles and practices. (Eds. Wyllie, E), 4th Edition. Lippincott Williams and Wilkins, Philadelphia. pp. 1185–1195.

Moore, M.S., DeZazzo, J., Luk, A.Y., Tully, T., Singh, C.M. and Heberlein, U. 1998. Ethanol intoxication in Drosophila: Genetic and pharmacological evidence for regulation by the cAMP signaling pathway. Cell 93: 997-1007.

Nichols, C.D. 2006. Drosophila melanogaster neurobiology, neuropharmacology, and how the fly can inform central nervous system drug discovery. *Pharmacol Ther*., 112: 677-700.

Nichols, C.D., Becnel, J. and Pandey, U.B. 2012 Methods to assay Drosophila behavior. *J Vis Exp (61)* e3795 doi: 10.3791/3795.

Nicolai, J., Vles, J.S. and Aldenkamp, A.P. 2008. Neurodevelopmental delay in children exposed to antiepileptic drugs in utero: a critical review directed at structural study-bias. *J. Neurol Sci*., 271: 1-14.

Olney, J.W., Wozniak, D.F., Jevtovic-Todorovic, V., Farber, N.B., Bittigau, P. and Ikonomidou, C. 2002. Drug-induced apoptotic neurodegeneration in the developing brain. *Brain Pathol.,* 12: 488-498.

Pendleton, R.G., Rasheed, A. and Hillman, R. 2000. Effects of adrenergic agents on locomotor behavior and reproductive development in Drosophila. *Drug Dev. Res.,* 50: 142-146.

Pizzi, W.J. and Jersey, R.M. 1992. Effects of prenatal diphenylhydantoin treatment on reproductive outcome, development, and behavior in rats. *Neurotoxicol Teratol*., 14: 111-117.

Rayburn, W.F., Gonzalez, C.L., Parker, K.M. and Christensen, H.D. 2004. Chronic prenatal exposure to carbamazepine and behavior effects on mice offspring. *Am J Obstet Gynecol.,* 190: 517-521.

Schmitz, B. 2006. Effects of antiepileptic drugs on mood and behavior. Epilepsia 47 Suppl 2: 28-33.

Sharma, C.S., Nema, R.K. and Sharma, V.K. 2010. Synthesis, anticonvulsant activity and in silico study of some novel amino acids incorporated bicyclo compounds. *J. Pharm. Sci.,* 2: 42-47.

Sokolowski, M.B. 2001. Drosophila: genetics meets behaviour. *Nat Rev. Genet.,* 2: 879- 890.

Sokolowski, M.B. and Hansell, R.I. 1983. Elucidating the behavioral phenotype of Drosophila melanogaster larvae: correlations between larval foraging strategies and pupation height. *Behav Genet*, 13: 267-280.

Soliman, G.A., Abla, A. and el, M. 1999. Effects of antiepileptic drugs carbamazepine and sodium valproate on fertility of male rats. *Dtsch Tierarztl Wochenschr*., 106: 110-113.

Stocker, R.F. 1994. The organization of the chemosensory system in Drosophila melanogaster: a review. *Cell Tissue Res*., 275: 3-26.

Ting, C.Y. and Lee, C.H. 2007. Visual circuit development in Drosophila. *Curr Opin Neurobiol*., 17: 65-72.

Todd, A.M. and Staveley, B.E. 2004. Novel assay and analysis for measuring climbing ability in Drosophila. *Drosophila Information Science*, 87, 101-107.

Truman, J.W., Taylor, B.J. and Awad, T.A. 1993. Formation of the adult nervous system. In: Development of Drosophila melanogaster. (Eds. Bate, M and Martinez Arias, A), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA. pp. 1245–1275.

Williams, G., King, J., Cunningham, M., Stephan, M., Kerr, B. and Hersh, J.H. 2001. Fetal valproate syndrome and autism: additional evidence of an association. *Dev Med Child Neurol*., 43: 202-206.

Zadrozniak, A., Wojda, E., Wlaz, A. and Luszczki, J.J. 2009. Characterization of acute adverse-effect profiles of selected antiepileptic drugs in the grip-strength test in mice. *Pharmacol Rep*., 61: 737-742.

****