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Interspecies Variation in Pupation Site Preference on Exposure to Different Anti Epileptic Drugs - A Study in Few Species of *Drosophila*

B. P. Harini

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

Abstract: ***The present work determine the acute adverse effects produced by commonly used conventional AEDs with respect to their larval pupation site preference in Drosophila species at different doses. Dose dependent action of different AEDs produced a maximal effect on behaviors of Drosophila species on exposure to phenytoin followed by valproiuc acid and carbamezepine which provides an efficient system to study genetic, neurological, and behavioral mechanisms. Such studies are useful for understanding the multiple effects on behavior. Larval pupation site preference is an important event in pre-adult development. Pupation site preferences of Drosophila larvae depend on species-specific cues. The present study revealed that the pupation site preference between species significantly varied on exposure to different AEDs and prefer media at large for pupation at high doses. D. melanogaster, D. ananassae and D. nasuta nasuta do not belong to same species group, but show similarity in their pupation site preference.***

Keywords: Drosophila species, Pupation site preference, Phenytoin, Valproic acid, Carbamezepine.

1. Introduction

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 (Bittigau *et al*., 2003); 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 (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 (Rayburn *et al*., 2004).

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). 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. 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).

The larval pupation site preference (PSP) is an important event in *Drosophila* pre-adult development, because the place selected by the larva can have decisive influence on their subsequent survival as pupae (Sameoto and Miller, 1968). *Drosophila* sensory systems contribute to detect, localize and provide information about the availability of food and chemical features of environments (Beltrami *et al*., 2012). The larval PSP has been analyzed by measuring the percentage of larva pupated at different sites *viz.,* cotton, glass and medium and revealed that most of the *Drosophila* species prefer media and a few species prefer glass and cotton for pupation (Vandal *et al*., 2003).

Drug induced changes in neural activities that cause complex behavioral changes are often drug induced illness (Nestler, 2005). Memory and learning in rats was affected by phenytoin including decreased radial maze (Tsutsumi *et al.*, 1998). Phenytoin also caused increased adrenaline and noradrenaline concentrations in response to stress in rats (Makatsori *et al.,* 2005) and increased hyper excitability in

monkeys (Phillips and Lockard, 1996).

The pupation height has been studied by measuring the distance a larva pupated above the surface of the food medium (Schnebel and Grossfield, 1992). Pandey and Singh (1993) and Joshi (1997) have noticed the effect of abiotic and biotic factors on pupation height in various species of *Drosophila* and concluded that developmental as well as gene environment interactions affects pupation site choice. Thus, total fitness is heavily influenced at the larval stage, and pupation site preference (Markow, 1979). Singh and Pandey (1993) have also reported that pupation height in *D. ananassae* is under polygenic control with a substantial amount of additive genetic variation.

Sokolowski and Hansell (1983) found positive correlation between pupation height and larval foraging behavior in *D. melanagaster*. Studies have shown that larvae are able to assess risks and modify their behavior to suit the environment in a way that increases the odds of survival and pupation position has an impact on survival (Riedl *et al.,* 2007). Singh and Pandey (1991) found intra and inter species variations in pupation height in three species *D. ananassae, D. bipectinata* and *D. malerkotliana*. Variations among different strains of the same species in pupation height can be attributed to genetic heterogeneity among strains.

The existence of genetically regulated behaviors is of great value for the adaptation of organisms. One aspect of the life cycle of *Drosophila,* the larval choice of suitable pupation sites has been the subject of different studies and in view of this behavior has on the subsequent pupal viability (Casares *et al.,* 1997). Difference in the choice of pupation sites by *Drosophila* in the laboratory have been proven to be under genetic control (Markow, 1979).

In light of the above studies the present work determine the acute adverse effects produced by commonly used conventional AEDs with respect to their larval pupation site preference 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*.

2. 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 compared to respective controls**.**

Larval pupation site preference

The AEDs were added to wheat cream agar media containing the above said doses and in the said *Drosophila* species were exposed. Virgin females and unmated males, separately collected were maintained for 5 days in order to age and then transferred to media containing drugs. 5 ml of media was placed in 25x100 mm sample tubes and a pair of flies was transferred to each vial. Flies were allowed to lay eggs on media supplemented with drugs and the number of eggs laid was recorded. Controls of different species were used for comparison. The vials were screened for the eggs to hatch, complete larval and pupal development. The PSP of the late third instar larvae which enter the wandering stage leaving the media was observed and recorded (Riedl *et al.,* 2007). The numbers of larvae pupated at different sites (cotton, glass wall and media) were counted and tabulated.

3. Results

The mean larval PSP in different species of *Drosophila* on exposure to different AEDs (Fig 1a). *D. melanogaster, D. ananassae* and *D. nasuta nasuta* control flies prefer to pupate on glass wall. The pupation on glass wall was highest at 5 mg/ml (47.7±0.94) in *D. melanogaster* and least at 15 mg/ml (26.3±3.32). The treated larvae of *D. ananassae* increased pupation on media (11.5±1.58 and 18.6±5.58) and decreased on glass wall (29.0±2.01 and 23.6±1.89) at 10 and 15 mg/ml when compared to control media and glass pupation. *D. nasuta nasuta* showed reduction at all the doses on glass wall; 28.1±3.72 at 5 mg/ml and 0.0 at 10 and 15 mg/ml. The pupation on media was increased in *D. nasuta nasuta* at 10 and 15 mg/ml (49.06±5.03 and 52.4±2.31) when compared to *D. melanogaster* and *D. ananassae*.

Mean larval PSP on exposure to VPA for *Drosophila* species is depicted in Fig 1b. *D. melanogaster* and *D. ananassae* pupated on glass wall at 0.2 mg/ml (46.6±1.71, 44.7±1.41) but decreased to 16.26±4.13 and 20.3±4.5 at high doses respectively. The pupation on media was highest (17.1±1.69) and (12.3±4.6) at 0.4 mg/ml in *D. melanogaster* and *D. ananassae* respectively. This indicates that the pupation on media was highest with increased doses. The pupation on glass wall was not found at 0.4 mg/ml of the larvae treated, whereas the media pupation was increased at 0.3 (38.2±3.46) and 0.4 mg/ml (42.5±3.86) in *D. nasuta nasuta*. The glass pupation was least in *D. nasuta nasuta* compared to *D. melanogaster* and *D. ananassae*.

On exposure to CBZ the mean PSP of *Drosophila* species had shown differences among control and treated (Fig 2c). The mean PSP on glass wall did not differ between treated, while slight increase on media (5.7±2.98) at 8mg/ml in *D. melanogaster*. The highest pupation on media was observed in *D. ananassae* and *D. nasuta nasuta* at all the doses (5.3±1.5, 8.3±0.4, 11.5±2.13) and (18.6±1.89, 35.2±1.75, 40.4±2.91). *D.nasuta nasuta* shows reduced pupation on glass wall at all the doses (16.3±3.32, 12.1±2.28, 6.7±1.7) respectively. *D. nasuta nasuta* has shown increased pupation on media when compared to *D. melanogaster* and *D. ananassae.*

4. Discussion

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).

Larval pupation site preference is an important event in pre-adult development. Pupation site preferences of *Drosophila* larvae depend on species-specific chemical cues (Beltrami *et al.*, 2012). The present study revealed (Fig 1a-c) that the pupation site preference between species significantly varied on exposure to different AEDs and prefer media at large for pupation at high doses. *D. melanogaster, D. ananassae* and *D. nasuta nasuta* do not belong to same species group, but show similarity in their pupation site preference *i.e.,* glass wall.

D. melanogaster showed reduced glass wall pupation at high dose (26.3±3.32) of PHT exposure while *D. ananassae* (29.0±2.0 and 23.6±1.89) and *D. nasuta nasuta* (0.0 and 0.0) at mid and high doses respectively. In case of *D. nasuta nasuta* glass pupation was observed only in control and low dose while pupation on media was highest at mid and high doses. Similar observations were found in other AEDs too.

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.

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.

5. Acknowledgement

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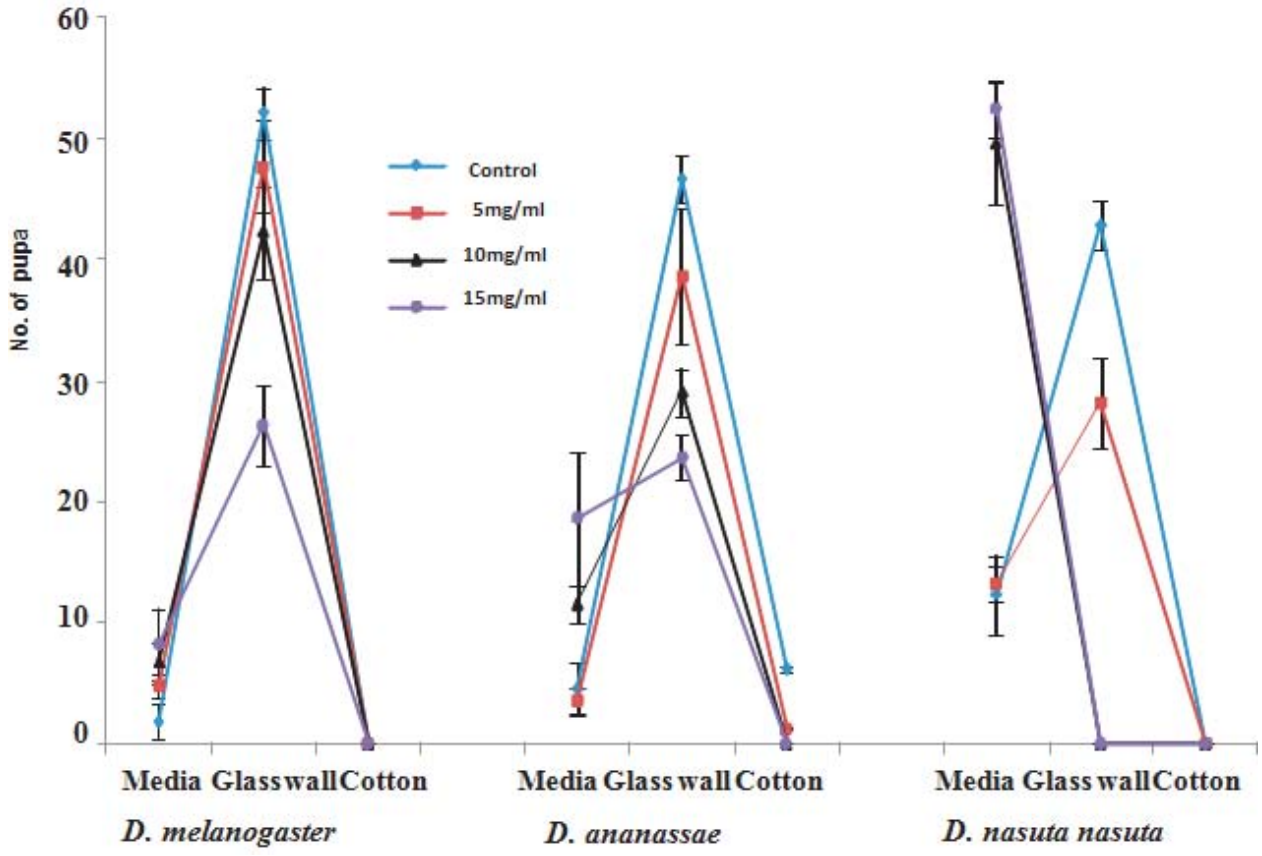
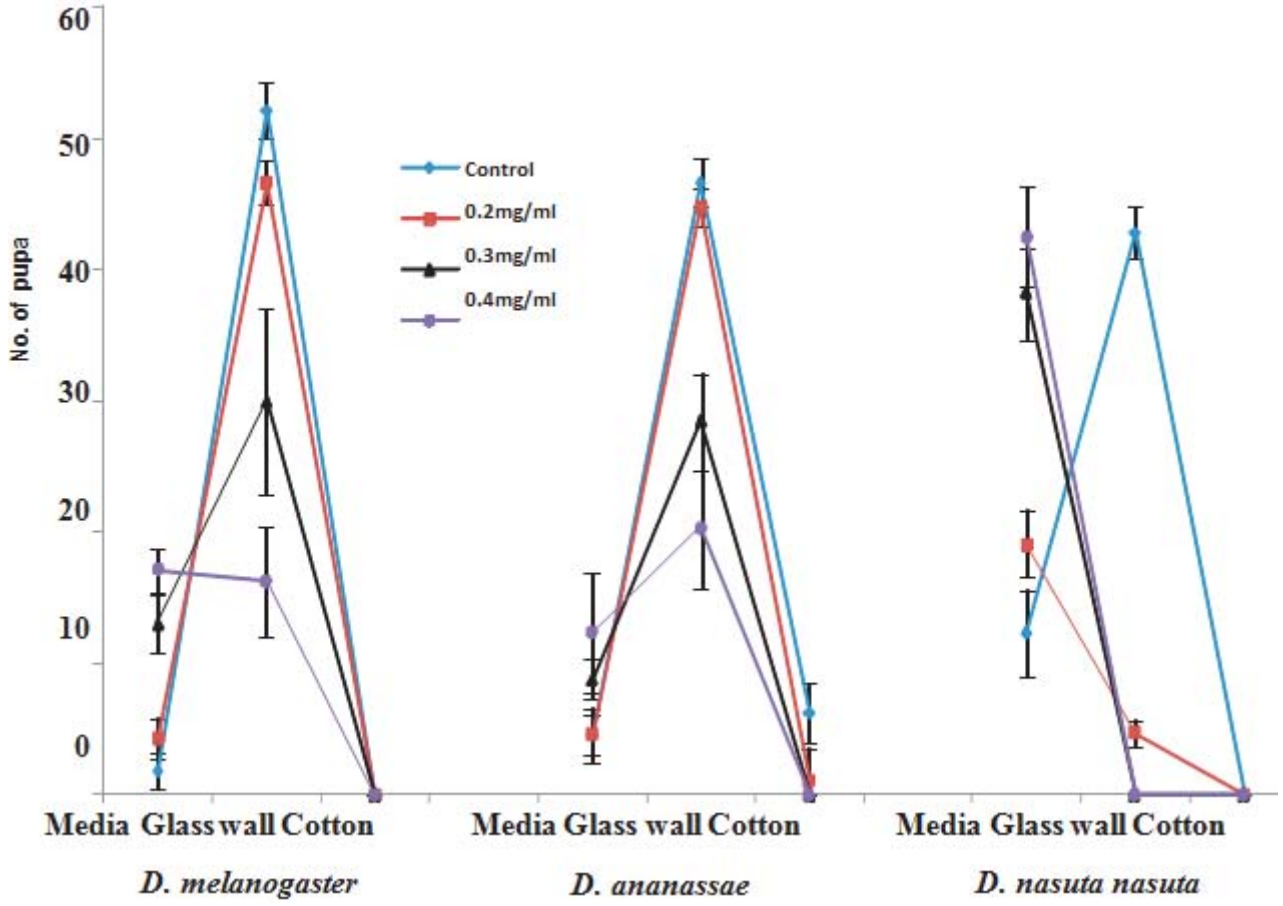
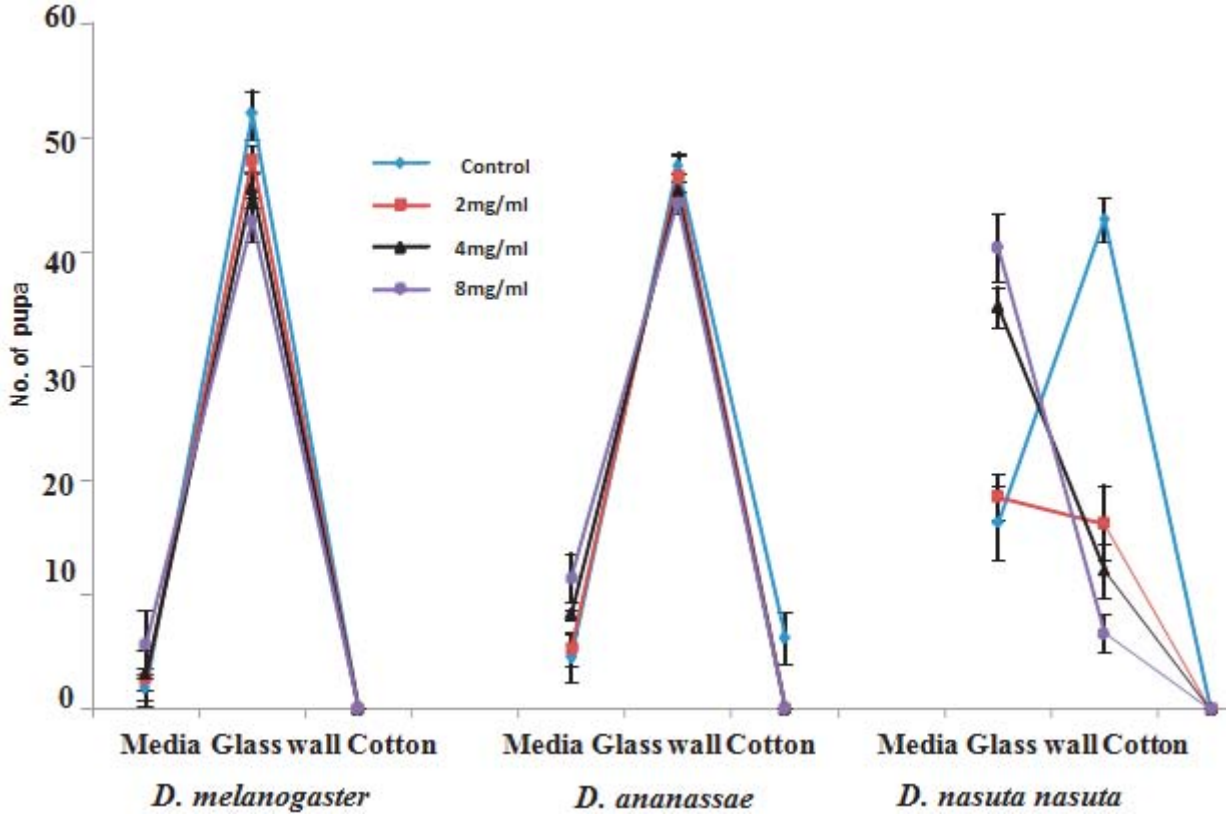


Figure 1a: Mean pupation site preference of *Drosophila* species on exposure to Phenytoin



Pupation sites of *Drosophila* species

Figure 1b: Mean pupation site preference of Drosophila species on exposure to Valproic acid



Pupation sites of *Drosophila* species

Figure 1c: Mean pupation site preference of Drosophila species on exposure to Carbamazepine