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# The Subsistence of Tonic-clonic Threshold in F1 and F2 Generation: A Study in Bang Sensitive Mutant of Drosophila on Exposure to Carbamizapine

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**ABSTRACT**

*The dose dependent action of carbamizapine an antiepileptic drug has played a pivotal role in diminishing the Seizure effect efficiently and the bangsensitive mutants provides an efficient system to study genetic, neurological, and behavioral mechanisms mediating these effects. Interestingly, the study has also revealed that carbamizapine do not have a prolonged threshold effect on the subsequent generation, the concentration of the dose is indirectly proportional to the tonic-clonic activity and has an important role in regulating behavior through metabolism; such studies should be useful for understanding the multiple effects on behavior and health. Despite the sources of complexity, the amount of research accomplished has pushed the fruit fly to the forefront of behavioral genetics research.*

**Keywords:** Drosophila bang sensitive mutant, Carbamizapine, Tonic-clonic activity, F1 & F2 generation

## INTRODUCTION

At the forefront of behavioral genetic research, D. melanogaster has provided important insights into the molecular, cellular and evolutionary basis of behavior [1]. Simple behavioral assays are widely applicable for studying the role of genetic and environmental factors on fly behavior on exposure to few AEDs [2]. Examining the effect known genetic mutations have on seizure susceptibility is one approach that may prove fruitful. This approach may be helpful in both understanding how different physiological processes affect seizure susceptibility and identifying novel therapeutic treatments. Seizure-like neuronal activities and behaviors in the fruit fly are described, as well as a set of mutations that exhibit features resembling some human epilepsy and render the fly sensitive to seizures. The genes responsible for seizure suppression are cloned with the goal of identifying targets for lead compounds that may be developed into new antiepileptic drugs [3].

Drosophila bang-sensitive (bs) mutants exhibit a stereotypic seizure and paralysis following exposure to

mechanical shock. In a physiological preparation, seizures and failures corresponding to the defective behavior are observed in response to high frequency stimulation. The amplitude of the stimulus necessary to produce bs behavior, or seizure threshold, varies with bs mutant and its gene dosage. In many respects, the bs defects are similar to those observed in mammalian seizure disorders [4].

Antiepileptic drugs (AEDs) have a variety of mechanisms of action which are reflected through different anticonvulsant activities and behavioral effects [5]. 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 [6]. The cognitive side effects of CBZ, PHT and VPA are comparable and associated with modest psychomotor slowing accompanied by decreased attention and memory [7].

The active metabolite carbamazepine epoxide is partly responsible for the mild cognitive and psychomotor effects attributed to CBZ [8]. 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 [9].

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" [10]. 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 [11].

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 Furthermore, at a physiological level the underlying neurotransmitter systems in the fly are conserved including serotonin, dopamine, GABA, glutamate and acetylcholine [12]. 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 [13]. The Mean Recovery Time (MRT) of seizure is an important event in Drosophila development. Drosophila sensory systems contribute to detect, localize and provide information about the availability of food and chemical features of environments [14].

The present is aimed to study the observable behaviour of valuable mutant of Drosophila bang-sensitive (BS) paralytic which is consistently found to be sensitive to seizure. In order to record for alterations in seizure susceptibility and the frequency of tonic-clonic activity of the parental stock and sustenance of seizure in the subsequent generation on treating with anti-epileptic drug i.e Carbamizapine.

## MATERIALS AND METHODS

The bang sensitive paralytic mutant were cultured on standard wheat cream agar medium in uncrowded culture condition at 22 ± 1ºC (rearing temperature) with a relative humidity of 80%. About fifty third instar larvae were picked up and starved for one day and thereafter placed in vial containing filter paper coated with a mixture of sucrose (5%) and a specific dose of carbamazepine (CBZ) drug (mg/ml) i.e 2 mg/ml, 4 mg/ml and 8 mg/ml for 24 hrs. Later 10 larvae per vials (parental stock) were placed in the vials containing wheat cream agar media for further development. About ten newly eclosed individual (6-8h old) flies were tested for the mechanical shock by vortexing individual flies for 10 seconds for tonic-clonic behavior with respect to drug paradigms. Similarly the control stock was also tested.

The resulting behavioral phenotype is complexed with distinguishable phase of seizure lasting several seconds. The mean recovery times of treated flies were examined in comparison to control cultures. Further the same set of stocks were reared for two generation to record the impact of sensitivity subsistence with respect to tonic-clonic behavior modulations and the mean Recovery time after a short stretch of mechanical shock.

The MRT has been studied by measuring the typical seizure lasting for several seconds, which is characterized by leg shaking, abdominal muscle contraction, wing flapping, wing scissoring and extension of proboscis and also time taken to be upright from ventro-dorsal to dorso-ventral positioning followed by initiation of locomotary activity.

## RESULTS AND DISCUSSION

The bang sensitive mutants have exhibited tonic-clonic effect in varied concentrations of carbamizapine (2 mg/ml, 4 mg/ml and 8 mg/ml) treated and as well in control stock. The MRT has increasingly significant difference (P>0.05) which last for more than 2minutes in control stocks. While in Parental stock the MRT is 111.2 ± 1.44, 99 ± 1.65 and 84 ± 1.98 minutes in low dose, mid dose and high dose respectively. Whereas in F1 generation eventhough the MRT is significant with P>0.030 and MRT is 149 ± 1.86 in control, and the values for low dose, mid dose and high dose are 73 ± 1.99, 70 ± 1.3362 ± 1.26. Interestingly the MRT has furthermore decreased in F2 generation with 51 ± 1.44, 42 ± 1.53, 34 ± 1.04 for low dose, mid dose and high dose respectively (Table 1).

Thus the mean recovery time has shown to increase with decreased concentrations in treated stock by more than 50% difference. While the MRT of toni-clonic effect has significantly increased in parental flies and subsequently found to diminish in the F1and F2 generations. Interestigly, on treating with carbamizapine tonic-clonic effect has been reduced from 90% to nearly 60%. The treated mutant flies have shown significantly faster recovery time upon treatment with CBZ.

The frequency of varied tonic-clonic events sensed by the mutants namely leg shaking, wing flapping, wing scissoring, abdominal muscle contraction and proboscis protrusion have shown to decrease with increased dose concentration and frequency was at its highest peak in control (Figure 1).

Drosophila has been used as a model organism in both medical and scientific research for over a century. The fly provides a very powerful genetic model system for the analysis of brain and behavioral disorders related to human disease: its brain is complex enough to make fly behavior highly interesting and relevant to humans. Despite having a much smaller genome (with approximately 180 million base pairs to a human's 3.2 billion), there is a surprising degree of gene homology [15,16]. Drosophila exhibits many of the same behaviors as humans viz., sleep, learn, remember, court and fight [17]. Further, Drosophila and humans have similar pharmacological and behavioral responses to a number of abused drugs [18-20].

In light of the above information, the present work determine the stereotypic Mean recovery time of seizure activity of bang sensitive mutants on exposure to anti-epileptic conventional drug carbamizapine. The present study has been assessed for the dose response relationship between AEDs and their behavior in epileptic mutant of Drosophila.

The genetic analysis remains the best means to define mechanisms and to begin the process of assigning the contribution of genes to behavior. Drosophila as an experimental organism dealt with the characterization of its behavioral responses was reported [20]. Two of the four life stages of Drosophila exhibit behavior: larva and adult. Today lot of information exists describing various aspects of Drosophila behavior in both larvae and adults. Sexual and non-sexual behavior of adults has been studied extensively [21].

The Drosophila flies were exposed to varying doses of antiepileptic drug for three days to determine its effect on behaviors. Few studies have addressed the issue of sexual activity in animals after AED treatment. Soliman et al. [22] reported that sexual desire was reduced in rats treated with either CBZ or VPA at very low doses. AEDs also influence sex steroid hormones via a centrally mediated effect, by altering gonadotropin secretion. 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 [23,24].

The observed mean value of tonic-clonic activity were dose dependent and significantly differs from the parental generation to successive F1 and F2 generation. The recovery time in control stock is greater when compared to the treated. The MRT has shown to increase with decrease dose and it is vise-versa with increase in dose. It is also noted that MRT observed in parental flies was significantly high when compared to subsequent generation i.e. F1 and F2. In addition to these observation, the frequency of varied tonic-clonic activity namely leg shaking, wing flapping, wing scissoring, abdominal muscle contraction and proboscis protrusion also decreased with increased dose concentration and frequency was at its highest peak in control.

**Table 1:** Mean Recovery Time (MRT) of tonic-clonic effect of bang sensitive paralytic mutant of Drosophila on exposure to carbamazepine in parental, F1 and F2 generations

| Generation | Concentration (mg/ml) | N* | MRT | P Value | % bss |
|---|---|---|---|---|---|
| Parental | 0 | 50 | 140 ± 1.02 | 0.013 | 95 |
| | 2 | 50 | 111.2 ± 1.44 | 0.002 | 83 |
| | 4 | 50 | 99 ± 1.65 | 0.010 | 77 |
| | 8 | 50 | 84 ± 1.98 | 0.001 | 63 |
| F1 generation | 0 | 50 | 149 ± 1.86 | 0.030 | 90 |
| | 2 | 50 | 73 ± 1.99 | 0.001 | 74 |
| | 4 | 50 | 70 ± 1.33 | 0.005 | 67 |
| | 8 | 50 | 62 ± 1.26 | 0.001 | 52 |
| F2 Generation | 0 | 50 | 142 ± 1.31 | 0.004 | 90 |
| | 2 | 50 | 51 ± 1.44 | 0.001 | 71 |
| | 4 | 50 | 42 ± 1.53 | 0.020 | 64 |
| | 8 | 50 | 34 ± 1.04 | 0.060 | 60 |

N* - Total no. of flies observed
MRT- Mean recovery time and ± Standard Deviation
P value – level of significance at 0.05
% bss – Percentage of flies prone to be bang sensitive to mechanical shock

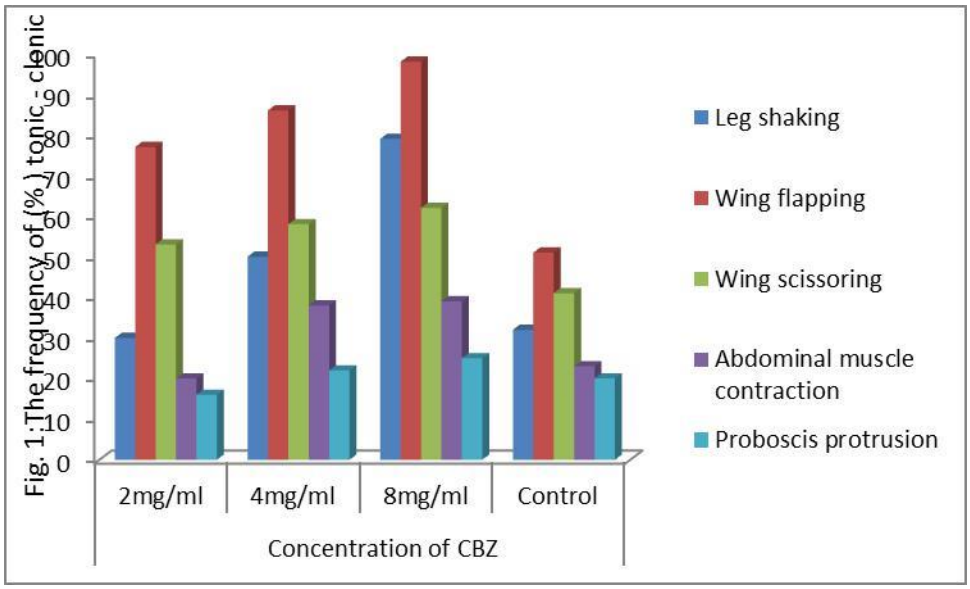


**Figure 1:** The frequency (%) of toni-clonic events sensed by the bang sensitive paralytic mutant of Drosophila on exposure to carbamazepine (CBZ)

## CONCLUSION

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.

Thereby the dose dependent action of carbamizapine an antiepileptic drug has played a pivotal role in diminishing the Seizure effect efficiently and provides an efficient system to study genetic, neurological, and behavioral mechanisms mediating these effects through generations. Interestingly, the study has also revealed that carbamizapine do not have a prolonged threshold effect on the subsequent generation and thus it has an important role in regulating behavior through metabolism; such studies should be useful for understanding the multiple effects on behavior and health.

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