Abstract

Systems biology is an emerging field that promises understanding of the entirety of processes that happen in a biological system, a long awaited objective of the biological sciences. Whereas molecules such as RNA and proteins are the focus of molecular biology, systems biology relates to entire system as a whole with molecules as components. The aim of systems approach is to comprehend the functioning of complex biological systems so that predictive models of human diseases could be developed. Because of enormous complexity of higher organisms, the focus of systems biology is currently on simpler organisms. It is in this backdrop that development of a *Drosophila* systems model of pentylenetetrazole (PTZ) induced locomotor plasticity responsive to antiepileptic drugs (AEDs) was undertaken. Chronic PTZ treatment is an established means to induce kindling in rodents. A model of brain plasticity, kindling involves recurrent activation of neural pathways that results in an increased susceptibility to evoked seizures and ultimately progresses to spontaneous seizures. Rodent kindling is widely used to model epileptogenesis. Epileptogenesis involves processes whereby structural and functional changes occur in the brain after an insult/ injury resulting in epilepsy. Kindling-like phenomena is also considered relevant in various neuropsychiatric conditions. AEDs are also used in treating, besides epilepsy, various other neurological and psychiatric conditions. Only a limited understanding exists at present as to how the initial electrographic seizure-induced changes in synaptic transmission and gene expression relate to permanent alteration in brain function induced by kindling. A systems level understanding of epileptogenesis is expected to facilitate development of novel
antiepileptogenic, disease-modifying, and neuroprotective agents. In the novel fly model that has been developed in our laboratory, seven days of PTZ treatment and seven days of subsequent PTZ discontinuation respectively cause a decrease and an increase in climbing speed of male Drosophila adults. The first part of the model, i.e., the chronic PTZ part, was validated using five known AEDs, namely, ethosuximide (ETH), gabapentin (GBP), vigabtrin (VGB), sodium valproate (NaVP) and levetiracetam (LEV). When applied concomitantly, NaVP and LEV suppress the development of locomotor deficit at the end of chronic PTZ phase, while ETH, GBP and VGB do not. In the time series microarray expression profiles of heads of flies treated with PTZ for 12 hrs (beginning phase), two days (latent phase) and seven days (behaviorally expressive phase) generated, Gene ontology (GO) biological process enrichment analysis of the differentially expressed genes at the above time points shows downregulation of transcription, neuron morphogenesis during differentiation, synaptic transmission, regulation of neurotransmitter levels, neurogenesis, axonogenesis, protein modification, axon guidance, actin filament organization, among other processes, in the latent phase and of glutamate metabolism, cell communication etc. in the expressive phase. Enrichment of Wnt signaling and other associated pathways in genes downregulated by PTZ was found after proteomic interactome based analysis and pathway overrepresentation analysis. Mining of available transcriptomic and proteomic data pertaining to established rodent models of epilepsy and human epileptic patients showed overrepresentation of epilepsy associated genes in the PTZ regulated set.
Given the potential value of the fly model in understanding long-term brain plasticity and in identifying drug targets, biomarkers, disease candidates and therapeutic agents, it was considered important to characterize the model further. Presented here is the further characterization of the model. It was carried out mainly in five parts. First, as PTZ withdrawal phase of the model was not studied earlier, the present study focused on understanding the behavioral pharmacology of PTZ withdrawal regime. All five AEDs mentioned above, i.e., ETH, GBP, VGB, NaVP and LEV, have been used in this analysis. Second, transcriptomic level characterization was carried out to understand genome scale expression changes associated with post-PTZ withdrawal regime. Third, transcriptomic effects of treatment with each of the five AEDs during post-PTZ withdrawal regime were analyzed to identify causal transcriptomic perturbations underlying PTZ withdrawal induced behavioral alteration. The full microarray data set has been deposited in the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) along with all the microarray information according to the Minimum Information About a Microarray Experiment (MIAME) guidelines developed by Microarray Gene Expression Data (MGED) society. Fourth, available protein interactome and miRNA-target databases were used to mine the transcriptome of the fly model towards identifying potential pathways. Fifth, transcriptomic effects of AEDs in normally grown flies were analyzed to understand drugs’ action under normal versus pathological conditions.

Each of the five AEDs was found to ameliorate development of climbing speed increase induced after PTZ withdrawal. It was unlike the previously described chronic
PTZ part of the model in which only two drugs out of five, NaVP and LEV, were found to be effective in ameliorating chronic PTZ induced development of climbing speed decrease. The pharmacology of kindling and post-kindling mechanisms in rodent models is also known to differ. The difference in behavioral pharmacology of chronic PTZ part and post-PTZ withdrawal part was thus significant.

Whole genome expression profiles of fly heads were next generated at three time points – 1st, 3rd and 7th day after PTZ withdrawal, i.e., on 8th, 10th and 14th day from beginning of PTZ treatment. Differentially expressed genes were obtained at the three time points. The genes showed enrichment of various categories of gene ontology (GO) molecular functions including transcription regulator and GTPase regulator activities in the behaviorally latent 10th day time-point. Upregulation of transcription regulatory and G-protein regulatory activities are already known in post-epileptogenesis in established rodent models. It was thus interesting to note the similarity between post-PTZ withdrawal fly model and post-epileptogenesis.

Since AEDs ameliorated development of locomotor abnormality post-PTZ withdrawal, it was of interest to examine if AEDs act through G-protein signaling and transcriptional regulation in the fly model. For this, microarray expression profiles of flies treated with AEDs post-PTZ withdrawal were generated. Flies were treated with PTZ for seven days, following which they were first treated for 3 days with ETH, GBP, VGB, NaVP or LEV and then with drug free media for next four days. Microarray expression profiles of heads were generated from flies collected on 10th
and 14th day from the beginning of PTZ treatment. Genes were predominantly downregulated by AEDs. Further analysis showed enrichment of various GO molecular functions in the differentially expressed genes. Whereas these functions included oxidoreductase and electron carrier activities found enriched in AED-untreated group, it was striking that unlike the latter none of the AED-treated group showed overrepresentation of transcription regulatory and GTPase regulator activities. Altogether, the transcriptomic analysis thus suggested that post-PTZ withdrawal induced alterations in transcription regulatory and GTPase regulator activities are ameliorated by AEDs. Given this, post-PTZ withdrawal in Drosophila was found to be a model relevant in post-epileptogenesis.

Given the evidence that post-PTZ fly model resembles post-epileptogenesis particularly in transcriptomic terms, the former was characterized to gain further insights into the underlying systems level perturbations. For this, the available protein interactome map and miRNA-target database of Drosophila was used. Based on overinteraction of genes and enrichment of miRNA-targets in the differentially expressed genes, perturbation in neuronal plasticity and axon guidance pathway was identified as potential mechanisms underlying post-PTZ withdrawal regime.

A centrally active drug may act differently under normal and pathological conditions. In this context, it was next examined if AEDs neutralizing effect on transcription regulatory and GTPase regulator activities detected above in the post-PTZ withdrawal model is due to downregulation of genes related to these categories by AEDs. For
this, microarray gene expression profiles of heads of normally grown flies treated with the AEDs were analyzed. The profiles for NaVP and LEV were already available. Expression profiling for rest of the AEDs, i.e., ETH, GBP and VGB, was freshly generated. Analysis of differentially expressed genes associated with AED treatment revealed enrichment of GTPase regulation related activities by ETH and GBP, not by VGB, NaVP and LEV. This suggested that VGB, NaVP and LEV may act differently under normal and pathological conditions.