

# Ethanol-induced G protein subunit expression changes in Dopamine receptor deficient *Drosophila*.

Oghenetega Umukoro, Olivia Corcoran, Andrew Thompsett and Stefano Casalotti

School Health Sport and Bioscience, University Of East London, Water Lane, Stratford, London. E15 5LZ. United Kingdom. [umukoro@uel.ac.uk](mailto:umukoro@uel.ac.uk)



## Introduction

Ethanol affects various neurotransmitter receptor systems and results in increased levels of extracellular dopamine in both the mammalian limbic system and the *Drosophila* CNS (Miguel-Hidalgo, 2009). Dopamine binding to its receptors leads to activation of G proteins which initiate or inhibit signalling cascades. Repetitive administration of ethanol is associated with changes in gene expression that could be responsible for alcohol induced addictive behaviours (Spanagel, 2009). In this work, we test in *Drosophila* the hypothesis that the expression of specific G proteins is altered by ethanol exposure. Such changes could cause a redistribution of G protein subtypes normally associated with specific receptor systems, altering signalling pathways and could account for addiction associated behaviours. The fruit fly *Drosophila melanogaster*, has proven to be a useful model system for identifying genes and pathways that mediate acute and chronic behavioural responses to ethanol (Devineni and Heberlein, 2010). *Drosophila* express both D1 and D2 like receptors which are associated with  $G\alpha_s$  and  $G\alpha_i$  subunits respectively (Hearn et al., 2002). In this study we have used a mutant in which the D1 receptor (subtype 2) is not functional to address whether ethanol-induced changes in G protein gene expression is affected.

## Materials and methods

*Drosophila Melanogaster* flies were reared in a 25° incubator in a 12 hour light/dark cycle with 60% humidity.

Table 1: Primers used in the study

Gα gene subunits	
<b>Gi</b>	
CG10060: G protein ai subunit 65A	
Forward primer	CGCGCAATGGGACGCCTGAA
Reverse primer	GCAGCAGGATGCCCTCGTCG
Primer length	106bp
<b>Gq</b>	
CG17759: G protein α 49B	
Forward primer	CAGCAGCACGCGAAAGCGTC
Reverse primer	GTCCCGGCGCAACTGCTTCT
Amplicon length	114bp
<b>Gs</b>	
CG2835: G protein sa 60A	
Forward primer	AGCAGGATATTCTCGGTGCCGT
Reverse primer	TTCCTACGCTCGTCCCGCTG
Amplicon length	118bp

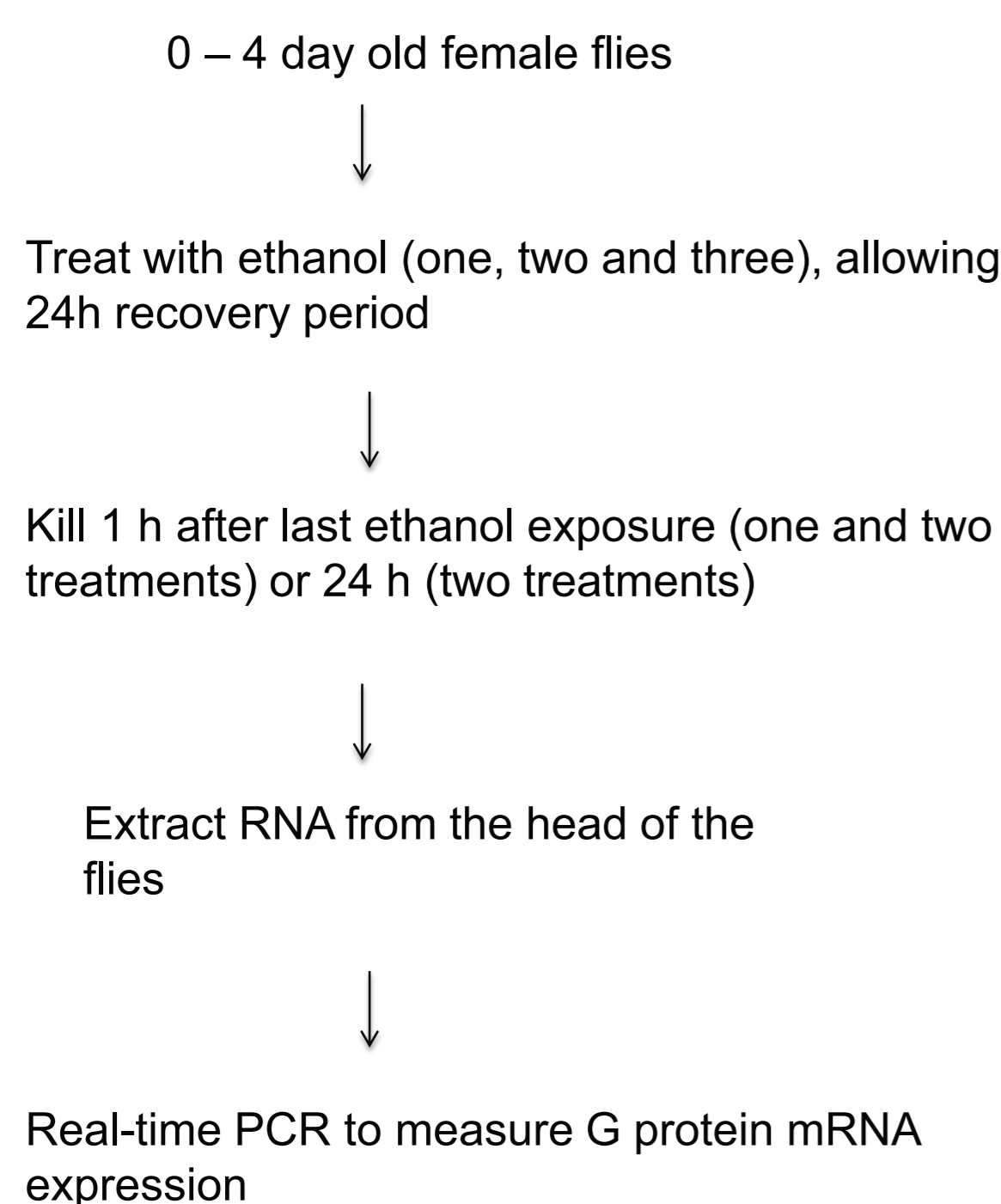


Figure 1. Scheme used to measure ethanol-induced G protein gene expression in *Drosophila*

## References

- (1)Devineni, A. V. and Heberlein, U. (2010) 'Addiction-like behavior in *Drosophila*', *Commun Integr Biol*, 3(4), pp. 357-9.
- (2)Hearn, M. G., Ren, Y., McBride, E. W., Reveillaud, I., Beinborn, M. and Kopin, A. S. (2002) 'A *Drosophila* dopamine 2-like receptor: Molecular characterization and identification of multiple alternatively
- (3)Miguel-Hidalgo, J. J. (2009) 'The role of glial cells in drug abuse', *Curr Drug Abuse Rev*, 2(1), pp. 72-82.
- (4)Spanagel, R. (2009) 'Alcoholism: a systems approach from molecular physiology to addictive behavior', *Physiol Rev*, 89(2), pp. 649-705

## Results

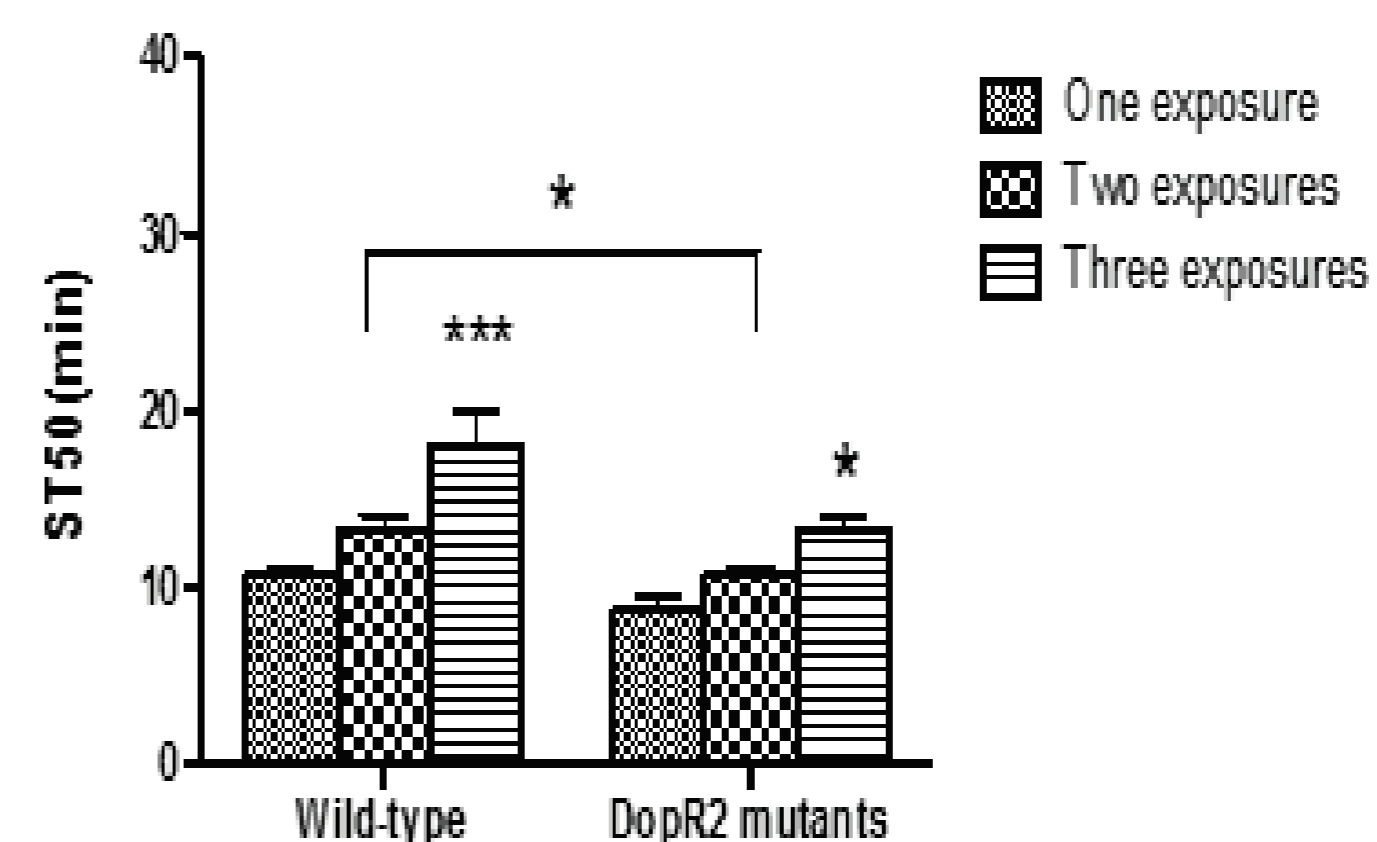
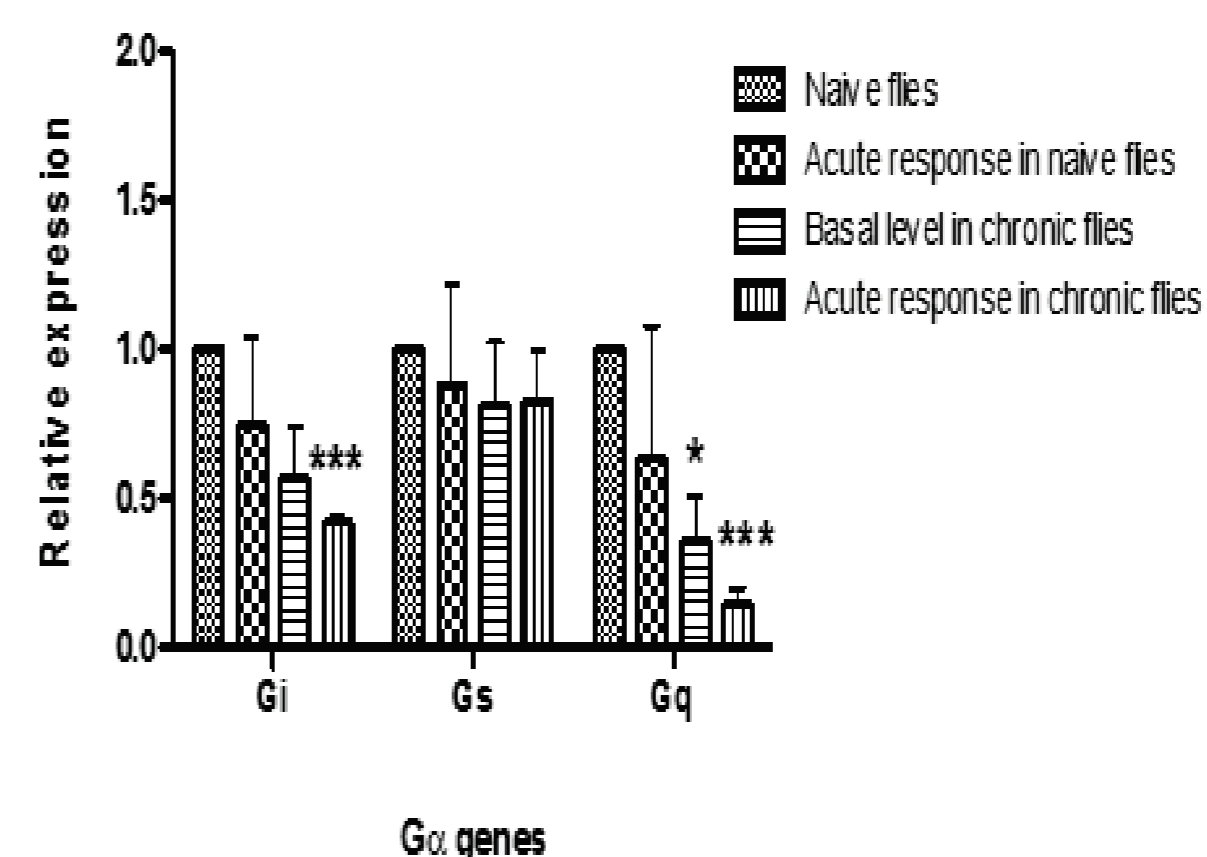
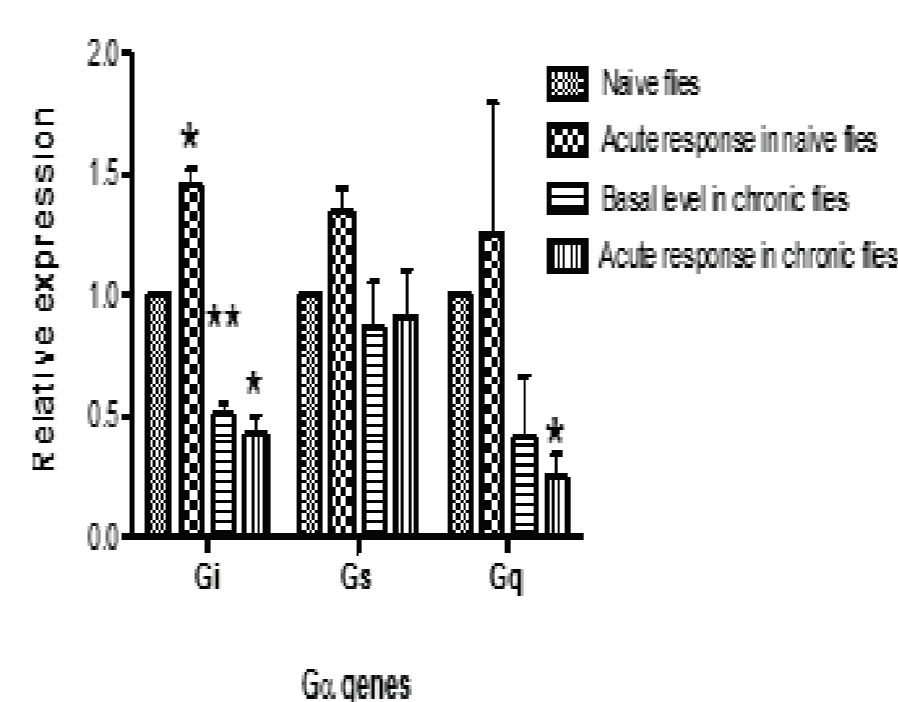


Figure 2. Tolerance induction in DopR2 mutants. DopR2 mutants do not express functional dopamine 1-like receptor 2. The time to 50% sedation – the time it takes half of the ethanol exposed flies to become stationary. DopR2 flies are more sensitive to the sedating effects of ethanol compared to wild type flies in the sensitivity assay, which is displayed as a reduced response in ST50.

## Dopamine mutants



## Gi mutants



## Gq mutants

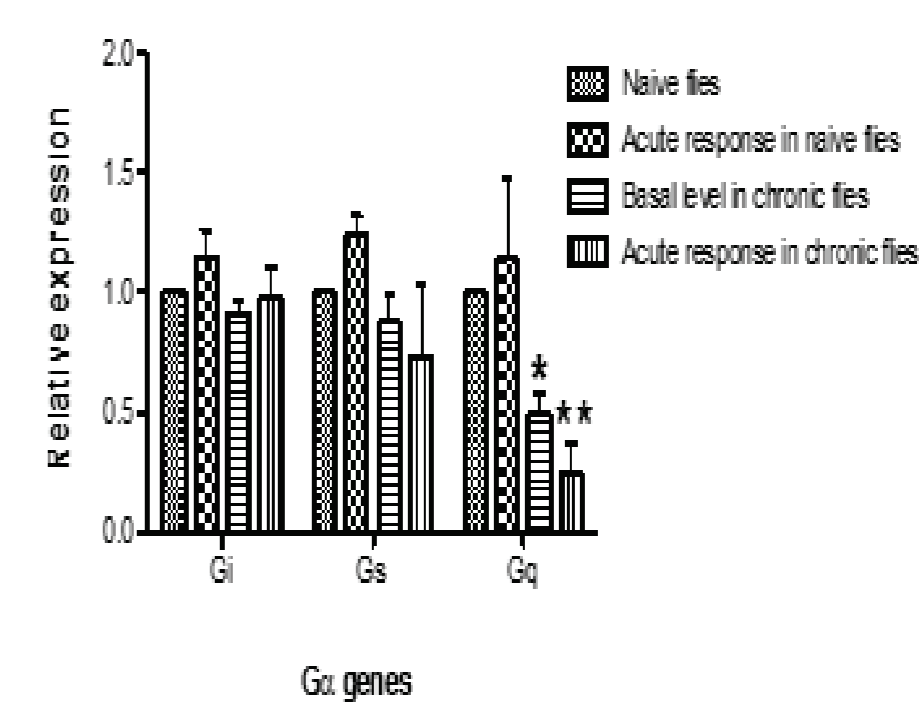


Figure 3. Relative Gα genes expression in the head of *Drosophila* mutants (Dopamine, Gi and Gq). Relative gene expression levels in the head of *Drosophila* mutants following treatment with 500µl of 100% ethanol once (acute response in naive flies), twice (basal level in chronic flies) and three times (acute response in chronic flies). Expression was normalised to β actin, and the means of mRNA levels are expressed relative to control ± standard error.

## Discussion

We have previously detected changes in G protein levels in the wild type sub-populations and we were interested to determine whether these changes were dependent on Dopamine receptors. In fact, we observed similar but larger changes in D1-type2 mutants (Figure 3) Expanding the research on G protein mutants, we confirmed further G protein expression changes and key points that we have observed are:

- $G\alpha_i$  and  $G\alpha_q$  (and not  $G\alpha_s$ ) are down regulated in chronically treated flies
- D1-type2 mutants and Gi mutants show the same response as wild type flies
- $G\alpha_q$  mutants show a reduction of only  $G\alpha_q$  and not  $G\alpha_i$

Therefore we propose that

- The expression of specific G protein subunits is affected by alcohol in *Drosophila*
- The changes in  $G\alpha_i$  are dependent on changes in  $G\alpha_q$

This finding suggests that further work is warranted to understand the full role of G protein gene expression in addiction, using *Drosophila* as a model.