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

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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 Gαs and Gαi 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	
Gi	CG10060: G protein αi subunit 65A
Forward primer	CGCGCAATGGGACGCCTGAA
Reverse primer	GCAGCAGGATGCCCTCGTCG
Primer length	106bp
Gq	CG17759: G protein α 49B
Forward primer	CAGCAGCACGCGAAAGCGTC
Reverse primer	GTCCCGGCGCAACTGCTTCT
Amplicon length	114bp
Gs	CG2835: G protein sα 60A
Forward primer	AGCAGGATATTCTTCGGTGCCGT
Reverse primer	TTCCTACGCTCGTCCCGCTG
Amplicon length	118bp

0 – 4 day old female flies

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Treat with ethanol (one, two and three), allowing 24h recovery period

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Kill 1 h after last ethanol exposure (one and two treatments) or 24 h (two treatments)

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Extract RNA from the head of the flies

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Real-time PCR to measure G protein mRNA expression

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

Results

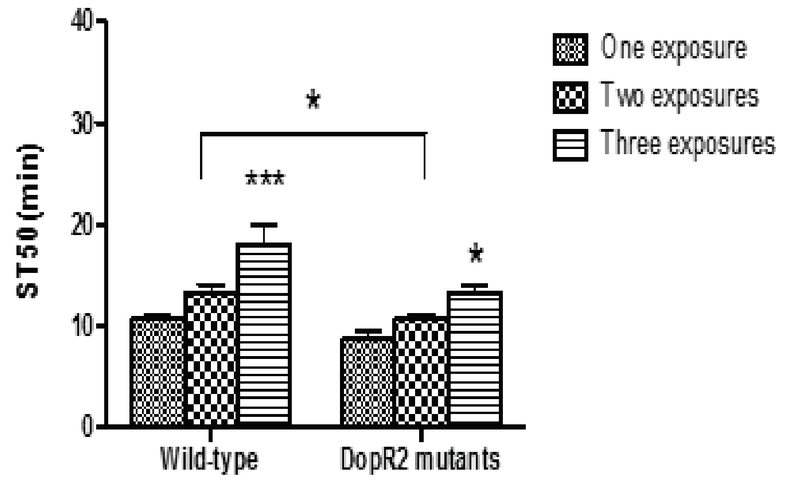

Figure 2. Tolerance induction in DopR2 mutants. DopR2 mutants do not express functional dopamine 1-like receptor 2. The time to 50% sedation – the time it takes half of the ethanol exposed flies to become stationary. DopR2 flies are more sensitive to the sedating effects of ethanol compared to wild type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

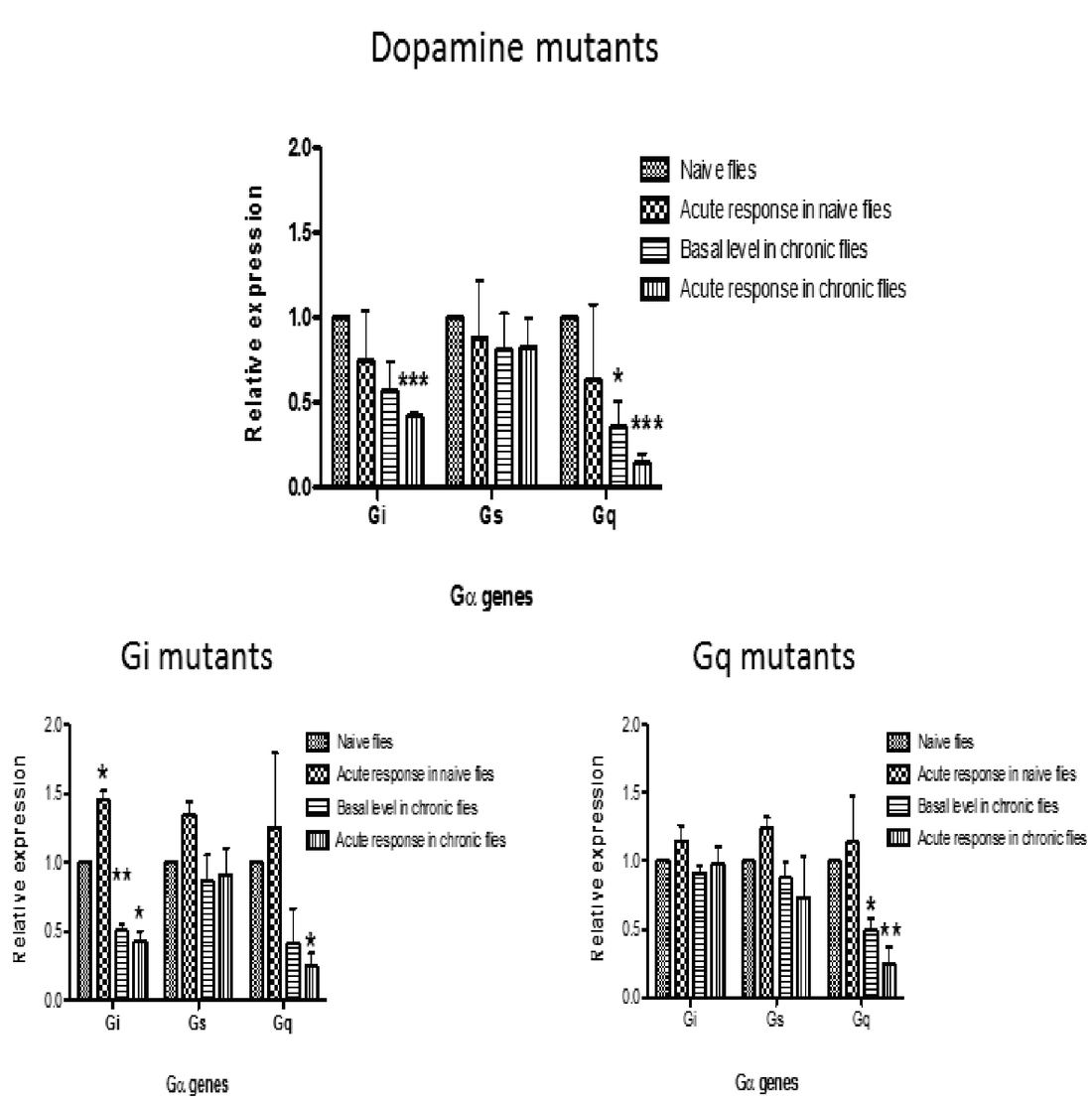

Figure 3. Relative Gα genes expression in the head of Drosophila mutants (Dopamine, Gi and Gq). Relative gene expression levels in the head of Drosophila mutants following treatment with 500µl of 100% ethanol once (acute response in naïve flies), twice (basal level in chronic flies) and three times (acute 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:

- Gαi and Gαq (and not Gαs) are down regulated in chronically treated flies
- D1-type2 mutants and Gi mutants show the same response as wild type flies
- Gαq mutants show a reduction of only Gαq and not Gαi

Therefore we propose that

- The expression of specific G protein subunits is affected by alcohol in *Drosophila*
- The changes in Gαi are dependent on changes in Gαq

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.

References

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