Glutamatergic hyperfunction in a C. elegans model of alcohol withdrawal

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A conceptual framework in which drug-induced withdrawal is rationalised as a behavioural representation of classic neuronal (synaptic) plasticity in key regions of the brain is facilitating understanding of alcohol withdrawal. Here we describe a project which applies this concept to the investigation of alcohol withdrawal in the model genetic animal C. elegans. To achieve this we have refined the understanding of the concentration-dependence of ethanol effects in C. elegans (Mitchell et al. 2007) and provided a paradigm for neuropeptide-dependent alcohol withdrawal (Mitchell et al. 2010). Currently we are using this model to interrogate the molecular genetics of the adaptive response to ethanol conditioning and in particular the relationship between neuropeptide signalling and glutamatergic hyperfunction.

Ethanol conditioning increases expression of a C. elegans ionotropic AMPA-like glutamate receptor, glr-2 (Kwon et al. 2004). To confirm this, age-synchronised populations of C. elegans were subjected to ethanol conditioning (3 to 6 hours, 250 mM) and the induction of ethanol withdrawal was measured in a navigational behavioural task, the 'food race', as previously described (Mitchell et al. 2010). Total RNA was extracted from withdrawn and control populations and real-time PCR carried out. Expression was normalized against actin-2.

After 6 hours ethanol conditioning there was a significant increase in the expression of glr-2 in wild-type worms (n=5; p<0.05). This induction paralleled the time-course of withdrawal behaviour as by comparison, after 3 hours conditioning (at which time-point withdrawal behaviour was not observed), there was no increase in glr-2 expression. The ethanol-induced increase in glr-2 expression was neuropeptide-dependent as it was not observed in egl-3 (a mutant deficient in neuropeptides). In a further experiment, a glr-2 mutant was subjected to ethanol conditioning and assayed for withdrawal behaviour in the food race. Preliminary data suggest that these mutants are less susceptible to ethanol withdrawal than wild-type controls.

These observations are consistent with previous reports that neuropeptidergic transmission sustains glutamate signalling in C. elegans (Mellem et al. 2002) and resonate with mammalian data which suggest an important role for glutamate hyperfunction in responses to chronic ethanol (Nagy et al. 2008). Currently we are further testing the hypothesis that ethanol conditioning triggers glutamate hyperfunction using optogenetic techniques to specifically activate C. elegans glutamatergic neurones and to compare glutamate-driven locomotor responses in ethanol conditioned and unconditioned animals in wild-type and egl-3 genetic backgrounds.

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