Poster L-13

Cerebellar Ethanol Sensitivity: A Central Role For Creb Transcription Regulation

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Short Abstract: The cerebellum is especially susceptible to ethanol toxicity. Inbred Long-Sleep (ILS) and Inbred Short-Sleep (ISS) mice, are a model for studying ethanol sensitivity. The C4.5 algorithm was applied to promoter sequences differentially expressed between ILS and ISS mice and indicates a central role for CREB transcription activity.

Long Abstract:
Inbred mouse strains, such as Inbred Long-Sleep (ILS) and Inbred Short-Sleep (ISS) mice, are useful for the study of the genetic basis of various drug addiction related phenotypes. For example, ILS and ISS mice differentially express a number of genes thought to be implicated in sensitivity to the effects of ethanol. Concomitantly, there is some evidence for a mediating role of cAMP/PKA/CREB signaling in aspects of drug addiction modeled in animals. Because the cerebellum is especially susceptible to ethanol toxicity, the current studies are focused on the cerebellum. The extent to which cAMP/PKA/CREB signaling impacts the differential expression of genes in ILS and ISS mouse cerebella is examined.
A training dataset for Machine Learning (ML) was generated from promoter region sequences (www.genome.ucsc.edu) of a set of genes known to be targets of CREB transcription regulation and a set of genes whose transcription regulations are potentially CREB-independent. For each promoter sequence, a vector of size 132, with elements characterizing nucleotide composition features was generated. The elements of the vector included a Boolean indicating whether or not the cAMP Response Element (CRE) was present, the number of nucleotide base pairs ("distance") between the CRE and the Transcription Start Site, and the "distance" between the CRE and the TFIID bind site. Four ML schemes were evaluated for their learning performance on the model created: a Decision Tree (J48, an implementation of the C4.5 algorithm), a Support Vector Machine (SMO), a Naïve Bayes classifier (NN) and a Multi-layered Perceptron (MLP) all available through the Weka ML workbench (http://www.cs.waikato.ac.nz/ml/weka/). Subsequently, a set of genes whose expressions have been previously determined [1] to be increased in ILS or ISS cerebella was identified and the CREB regulation status of each member predicted using the ML scheme C4.5.
RESULTS: Of the four ML schemes evaluated, the C4.5 Decision Tree algorithm had the lowest predicted error rate (as per the Ten-fold Cross Validation technique). The two classes to which the ML schemes assigned training and test genes were "CREB Regulated" and "Not CREB Regulated". On an independent test set of 21 genes of known CREB regulation status, C4.5 correctly classified 95.2% of instances with an F-measure of 0.97.
Additionally, all four genes determined by two independent microarray platforms [1] to be upregulated in the ILS cerebellum were determined by C4.5 to be transcriptionaly CREB regulated. The platforms were the Affymetrix (Santa Clara, CA) platform Mouse Expression Set 430 (MOE430) and the cDNA arrays NIA15K manufactured at the University of Colorado's School of Medicine. Similarly of all four genes upregulated by both platforms in

the ISS cerebellum were deemed CREB regulated. Also, 92% and 89% of a cross-section of other upregulated (as per the MOE430 platform) cerebellar genes in ILS and ISS mice, respectively, were deemed to be “CREB regulated”.
In another experimental model, a third class, “Nrf2 Regulated”, was introduced. Thus the classes to which genes were assigned were: “Nrf2 Regulated”, “CREB Regulated” and “Not CREB Regulated”. The C4.5 algorithm again assigned all four genes deemed by both the MOE430 and the NIA15K platforms as upregulated in the ILS mouse cerebellum to the “CREB Regulated” class; it also assigned all four genes deemed by both platforms as upregulated in the ISS mouse cerebellum to the “CREB Regulated” class. Furthermore, using this three-class model, the C4.5 algorithm assigned to the “CREB Regulated” class 81% and 80% of a cross section of genes determined to be upregulated on the MOE430 platform in ILS and ISS mouse cerebella respectively.
The stretch of nucleotides between the cAMP Response Element (CRE) and the transcription start site and the stretch between the CRE and the Transcription Factor II D bind site were identified as the most important determinants of a gene’s CREB regulation status. With the notable exception of the latter stretch, CREB targets have relatively high levels of nucleotide bases with strong Hydrogen Bonding.
The novelty of these ML-driven findings lies in their suggestion that ethanol sensitivity, as it relates to the cerebellum, is largely dependent on CREB transcription activity.

1. MacLaren EJ, Sikela JM. (2005) Alcohol Clin Exp Res. 29:1568-1579.