Error Correction and Clustering Gene Expression Data Using Majority Logic Decoding

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Abstract *Microarrays allow researchers to simultaneously measure the expression of thousands of genes. They give invaluable insight into the transcriptional state of biological systems, and can be important in understanding physiological as well as diseased conditions. However, the analysis of data from many thousands of genes, from only a few replications is very difficult.*

We have devised a novel method of correcting errors in microarray experiments, that also clusters genes into groups, and categorizes their measurements into coarse divisions, suitable for discrete techniques for reverse engineering. These techniques are based on finite fields and algebraic coding theory. We test these new techniques on a data set obtained from behavioral training experiments on rats, and identify two novel genes that may be involved in learning and memory.

Keywords: microarray, error-correction, discrete methods

1 Introduction

cDNA microarrays are a technique for measuring the abundance of messenger RNA from many thousands of genes simultaneously in an inexpensive experiment [1]. They are used extensively for diagnostic purposes, and the data they allow researchers to collect have permitted the study of genome wide interactions among genes. The analysis of microarray data, however, is a difficult task, proving a fruitful area of research in numerous fields. An extensive review is available in [2].

1.1 Clustering

Clustering of gene expression measurements is an important step in many analysis, most early microarray work performed hierarchal clustering, where genes are successively agglomerated into groups by selecting the two clusters whose average expression values are closest [3]. It is typical to first cluster genes before trying to determine the gene regulatory network by reverse engineering. Clustering helps reduce the computational resources required to analyze microarray data sets by grouping together many separate genes that demonstrate similar patterns of expression [4]. It also can help in determining common functionality or common regulatory elements of genes which cluster together [5].

1.2 Genetic network models

As early as 1969, Stuart A. Kauffman [6] (see [7] for a detailed review) proposed the far-reaching and important idea of using Boolean logic, the logic of computers, to produce and gain insight into the logic of genes. The invention of cDNA microarrays brought a resurgence of interest in these Boolean genetic network models.

1.3 Boolean models and the reverse engineering problem

A series of papers in 1998, 1999 and 2000 defined Boolean network models, reverse engineering, and proved interesting results on the number of experi-

ments required to completely define a Boolean network.

Taking the model definition from [8], for example, we can describe a Boolean genetic network as a directed graph consisting of N nodes numbered $1, 2, \ldots, N$, such that for each node n there is an associated Boolean function f_n. An edge from a node to another represents an influence of the first node on the expression of the second.

In [9] Akutsu *et al.* proved lower and upper bounds on the number of gene perturbation experiments required to completely determine a Boolean gene network. The results are discouraging, since in the general case, the problem is shown to be NP-complete. However, in [10], an efficient algorithm for determining the gene network from a set of input-output pairs is developed, assuming that each gene has an indegree in the directed graph that is at most three. This restriction corresponds to saying that at most three genes have an influence on the expression of the target gene. Further research proceeds on the assumption that this indegree is bounded by a small constant. In [4] it is shown that a gene network will be recovered with high probability in only $O(\log n)$ experiments if the indegree is at most two. The research in [8] provides an iterative procedure for selecting genes to perturb while determining a genetic network such that the uncertainty in the specification of the model is reduced. After this series of papers, work on these Boolean models was mostly discontinued, biologists objected to the simplicity of the Boolean representation of genes.

It is also important to note that all of these Boolean network papers leave unspecified the manner in which gene expression measurements are converted to Boolean values. For example, in [8] the authors simply say that gene values will be approximated as high or low and represented by the values 1 or 0.

1.4 Partial enumeration

In the Boolean network models, reverse engineering via partial enumeration of functions as described in [10, 9] requires limiting the number of inputs to each genetic function, usually assuming that between 2 to 4 genes affect the expression of a given gene. This requirement for computational tractability directly conflicts with the evidence that transcriptional networks for higher organisms are significantly more complex [11, 12], with even yeast having up to 10 or more transcription factors influencing the expression of a single gene [13].

1.5 Finite Dynamical Systems

Boolean networks have 2 limitations: they can only represent genes as "on" or "off", and they limit the nature of the gene interaction network to ensure computational tractability.

Finite dynamical systems (FDS) are a broad class of models that consist of a pair $(\mathrm{GF}(p^n), f)$. FDS represent the state of a system as a value over the finite field $\mathrm{GF}(p^n)$, and the state of the system evolves over time by iterating a fixed function $f : \mathrm{GF}(p^n) \to \mathrm{GF}p^n$. The function f applied to the current state yields the new state of the system. A particular type of finite dynamical system was developed in [14] for the simulation of computer systems, and later adapted for genetic regulatory networks [15].

These models allow for a richer variation of gene expression levels, and remove the restrictions on the degree of the genes. Several alternative representations and techniques for polynomial models over finite fields have been developed [16, 17, 18], and [19] demonstrates that these polynomial models are equivalent to those described in [14, 15].

This research lead to a series of techniques for error-correction, clustering, and reverse engineering [20] based on finite fields.

1.6 Microarray experiments

The microarray studies described here focused on one cognitive task, conditioned taste aversion (CTA), as a model system for gene expression profiling. CTA is an associative aversive conditioning paradigm in which pairing gastrointestinal malaise (induced by lithium chloride, LiCl, the unconditioned stimulus) with prior exposure to a novel taste (the conditioned stimulus) may create a strong and long lasting aversion to the novel taste.

CTA lends itself as an excellent model system to study the dynamics of gene regulation in learning and memory because it is a single trial associative learning paradigm, which involves discrete regions in the brain, including selected amygdala nuclei [21, 22].

1.6.1 Behavioral training

Behavioral training of rats in the CTA task prior to collection of the microarray data used for our experiments was done as described in [23].

1.6.2 Microarray measurements

The gene profiling experiment was replicated five times. Four animals were used per condition for each replicate. Thus, a total of twenty rats were used per condition. Animals were sacrificed by decapitation at 1, 3, 6, and 24 hours after conditioning and amygdala enriched tissue punches were obtained for RNA isolation. Hybridization, image capture and analysis was similar to the procedures described in [24]. The data set thus obtained (CTA data set) is described in [25]. In summary, the data has two controls, the pre-treatment group and the one hour saline group, and four time points, 1, 3, 6, and 24 hours after conditioning. Each array has 1185 genes, and we have 5 biological replicates of each array.

2 Methods

The methods describe here were developed for the purpose of analyzing the CTA data set, but are sufficiently general to analyze any equivalent data set.

2.1 Error Correction and Clustering

We have devised a scheme for detecting and correcting errors using discretized data.

Here we apply our technique to data from gene A01a in the CTA data set described in Section 1, to illustrate the method:

Pre	Sal	1 h	3 h	6 h	24h
0.172	0.099	0.176	0.142	0.062	0.152
0.274	0.168	0.126	0.114	0.104	0.276
0.003	0.119	0.552	0.178	0.193	0.114
0.114	0.139	0.6	0.311	0.179	0.181
0.04	0.006	0.172	0.103	0.036	-0.047

Each row is a repetition of the microarray experiment. Columns represent the measurements of the genes. Pre and Sal are the pretreatment (time 0) and injection with saline solution controls.

2.2 Averaging

The first step in the analysis is to average the expression across repetitions.

average	0.12	0.11	0.32	0.17	0.12	0.13

We also average our control columns to obtain a control value of 0.115.

We compute an epsilon value, such that either the 1 h or 24 h columns are within the range of control +/- epsilon. In this case, the epsilon is 0.022.

2.3 Discretization

We proceed to discretize each repetition by comparing each column to the control +/- epsilon. We illustrate for repetition 1:

Pre	Sal	1 h	3 h	6 h	24h
0.172	0.099	0.176	0.142	0.062	0.152

The control for this repetition is $(0.172 + 0.099)/2 = 0.1355$, epsilon is fixed for all our tests at 0.022. We now call a column "+" if its value is greater than the control + epsilon, "-" if is is less than control - epsilon, and "0" otherwise.

Pre	Sal	1 h	3 h	6 h	24h
+	-	+	0	-	0

Repeating for the remaining repetitions yields;

Pre	Sal	1 h	3 h	6 h	24h
+	-	+	0	-	0
+	-	-	-	-	+
-	+	+	+	+	+
0	0	+	+	+	+
0	0	+	+	0	-

2.4 Majority logic decoding

We now obtain a consensus for each column by majority logic decoding, 3 or more occurrences of the same symbol in a column indicate that symbol is the consensus. If no consensus is obtained, we indicate "?".

	Pre	Sal	1 h	3 h	6 h	24h
consensus	?	?	+	+	?	+

2.5 Discretizing against averaged controls

The above procedure is very sensitive to the value of the controls. Errors in the controls can skew the entire set of calls. We devised an alternate method of discretization that replaces the control value for each row by the average of the control value for all the rows. In our case this average control is 0.113. The discretization of the repetitions using this average control yields the following values, which we summarize with this consensus versus average control (cvac):

	Pre	Sal	1 h	3 h	6 h	24h
	+	0	+	+	-	+
	+	+	0	0	0	+
	-	0	+	+	+	0
	0	+	+	+	+	+
	-	-	+	0	-	-
cvac	?	?	+	+	?	+

2.6 Discretizing the average

We also compute the discretization of the average values of each column, using the control 0.113 and the epsilon 0.022:

	Pre	Sal	1 h	3 h	6 h	24h
average	0.12	0.11	0.32	0.17	0.12	0.14
calls	0	0	+	+	0	0

2.7 Error correction

We now enter an error correction phase, we seek out outliers in the data of the columns and remove them, and recompute the average, controls, and epsilon.

Pre	Sal	1 h	3 h	6 h	24h
—	0.099	0.176	0.142	—	0.152
—	—	0.126	0.114	0.104	—
0.003	0.119	—	—	0.193	0.114
0.114	0.139	—	—	0.179	0.181
0.04	—	0.172	0.103	—	—

With these outliers deleted from our data we now have new averages, control and epsilon values:

	Pre	Sal	1 h	3 h	6 h	24h
average	0.052	0.119	0.158	0.12	0.159	0.149
control	0.086					
epsilon	0.063					
calls	0	0	+	0	+	0

2.8 Consistent calls

We are now ready to produce a consistent set of calls for the gene. A set of calls is consistent if the following conditions are met:

1. at least two of the above set of calls agrees in the last 4 columns of data (1 h, 3 h, 6 h, and 24h)
2. either the 1 h or the 24 h columns is a "0"
3. across the last 4 columns of data, the column exhibits the consecutive zeros property (*i.e.,* values do not oscillate between "0" and "+" or "-")

As an example, the set of calls for A01a are:

	1 h	3 h	6 h	24h
consensus	+	+	?	+
cvac	+	+	?	+
average calls	+	+	0	0
new calls	+	0	+	0

These calls are not consistent, and this gene is removed from further examination. Together, the procedures we developed and the consistency criteria try to capture biologist's intuitions on the nature of gene expression changes.

3 Results

We have performed the analysis described above on the CTA data set described in Section 1.6.2. In this data set, there are 127 consistent genes, which we divide into clusters by grouping together the genes that have the same set of calls in the 1 hour through 24 hour timepoints. This results in the 23 clusters shown in Table 1.

Table 1: Consistent genes clustered by the error correction procedure.

Cluster	Gene coordinate
- 0 0 0	A05f, A12k, A14g, B07m, B07n
0 + + +	B01n, B04i, B05l, B06j, B06l, C10l, D01j, D10l, E01k, E03j, E09c, E10e, E13i, E13l, F01e
+ 0 0 0	B07e, D04f, D09l
- - 0 0	A02m, A14e, B03i, C08i, F08c, F13e
- - + 0	C14k, E03l
0 0 + +	B13n, D14g, E06i, E10m, E11l, E13e, E14d
- + + 0	B06m, E02i
0 0 - -	A05n, D02a, F12e
0 - + +	B04j, E10l, F11j
+ - - 0	C01f, F01a
0 - + -	A11d, C09m
- + - 0	D12a
0 + - +	D09i
- - - 0	A02l, A03h, A09c, B10l, C02m, C04d, C04f, C06e, D02b, F02b, F03c, F09n, F11e
+ - + 0	B13a, F02a
0 0 0 -	A10l, C08a, C14g, C14l, D13e
+ + 0 0	A08l, B08b, C08j, F11k
+ + - 0	D04l
0 0 0 +	A07i, B09h, C10c, D08n, E03m, E04i, E13h, E14f
0 - - +	C01e, F12j
0 - - -	B05b, C13a, C13i, C14n, E02e, F04l, F06a, F11l
- + 0 0	A12m
+ + + 0	B01i, B07f, B10c, B10d, B14h, D08f, D09e, E03i, E14k, F02n, F05k, G15

A particulr focus of interest in our studies was the identification of genes regulated by the transcription factor CREB (cAMP Responsive Element Binding protein), which is known to play important roles in memory formation [26]. We focused on the expression of both CREB and other genes with similar patterns of expression in order to detect changes in gene expression paralleling CREB's expression. CREB binds to a DNA element called cAMP-response element (CRE) in the promoter region of its target genes, and in conjunction with a co-activator promotes the initiation of their transcription [27].

There are two genes in our dataset that bind CRE, A05h and A05l, these genes are described below:

Coord.	Accession	Description
A05h	U38938	activating transcription factor 2 (ATF2); cAMP response element DNA-binding protein 1 (CREBP1)
A05l	X14788	cAMP-responsive element-binding protein 1 (CREB1)

The coordinate column is a unique identifier for the spot on the Clontech arrays, the accession number is Clontech's assignment of a gene in the Genbank nucleotide database to this entry. Of the two CREB genes on the arrays, the gene most associated with learning and memory processes is A05l, or Creb1. The discretization of the average expression of A05l yields "000+", therefore we focused on the cluster labeled "000+". The calls for these genes represents no change over the 1, 3, and 6 hour time points, followed by upregulation at the 24 hour timepoint. This cluster consists of genes whose expression most closely matches the expression profile of Creb1. We investigated the genes in this cluster in depth, retrieving the gene information and sequence from the Ensembl Genome Browser version 32 [28].

Coord.	Accession	Description
A07i	L24388	galactosyltransferase-associated protein kinase (GTA); CDC2-related protein kinase (CDC2L1)
B09h	L10362	synaptic vesicle protein 2B
C10c	L33869	ceruloplasmin (CERP; CP); ferroxidase
D08n	X63255	N-methyl-D-aspartate receptor subtype 1 (NMDAR1; NR1); glutamate receptor subunit zeta 1 (GRIN1)
E03m	M29712	melanin-concentrating hormone (PMCH; MCH)
E04i	V01228	calcitonin
E13h	M20713	guanine nucleotide-binding protein G(K) alpha 3 subunit (G(I) alpha 3 (GNAI3)
E14f	X06890	ras-related protein RAB4A

From Ensembl we obtained genomic sequence for each of these genes, 1020 base pairs starting 800 base pairs upstream of the transcription start site. These sequences were then submitted to TESS [29] to search for transcription factor binding sites. We look for the CRE element, a DNA sequence that is the target site for CREB. Genes that have CRE in their upstream region are potential targets of regulation by CREB.

Based on our findings we focused on two specific genes: E03m or Pmch (pro-melanin-concentrating hormone) and E04i or Calca (calcitonin/calcitonin-related polypeptide, alpha). Both genes have CRE elements in their upstream regions. According to the Rat Genome Database [30], Pmch is a cyclic neuropeptide that induces hippocampal synaptic transmission. Pmch also seems to have an effect on appetite or metabolism [31] and anxiety [32], and promotes synaptic transmission in the hippocampus [33]. Calca is principally a vasodilator, but seems to have a role in axonal regeneration or synaptogenesis [34]. Thus, these genes exhibit a pattern of expression consistent with the expression of Creb1, have CRE elements upstream of their transcription start site, and seem to have a role in strengthening or creating new synapses.

4 Discussion

We have developed a method for error correction of microarray experiments. The technique produces a clustering of genes and describes each gene as unchanged, upregulated, or downregulated, in accordance to biologists natural description of expression levels. We applied these techniques to a microarray data set derived from a CTA experiment in rats, looking for genes that may be important in learning and memory processes. We found two genes, Pmch and Calca, that share an expression pattern with CREB, contain CRE in their upstream regions, and have demonstrated function related to synaptic plasticity. Pmch and Calca are strongly implicated as important genes for the formation of memories. We are now actively seeking confirmation of these genes' role in CTA and of their regulation by CREB as a result of CTA training.

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References

[1] M. Schena, D. Shalon, R. W. Davis, and P. O. Brown, "Quantitative monitoring of gene expression patterns with a complementary DNA

microarray," *Science*, vol. 270, no. 5235, pp. 467–470, Oct 20; 1995.

[2] H. de Jong, "Modeling and simulation of genetic regulatory systems: A literature review," *Journal of Computational Biology*, vol. 9, no. 1, pp. 67–103, 2002.

[3] M. Eisen, P. Spellman, D. Botstein, and P. Brown, "Cluster analysis and display of genome-wide expression patterns," *Proceedings of National Academy of Science*, vol. 95, pp. 14 863–14 867, 1998.

[4] T. Akutsu, S. Miyano, and S. Kuhara, "Identification of genetic networks from a small number of gene expression patterns under the Boolean network model," *Pacific Symposium on Biocomputing*, vol. 4, pp. 17–28, 1999.

[5] P. D'haeseleer, S. Liang, and R. Somogyi, "Genetic network inference: from co-expression clustering to reverse engineering." *Bioinformatics*, vol. 16, no. 8, pp. 707–726, Aug 2000.

[6] S. A. Kauffman, "Metabolic stability and epigenesis in randomly constructed genetic nets," *J. Theor. Biol.*, vol. 22, pp. 437–467, 1969.

[7] ——, *The Origins of Order*. New York, Oxford: Oxford University Press, 1993.

[8] T. E. Ideker, V. Thorsson, and R. M. Karp, "Discovery of regulatory interactions through perturbation: Inference and experimental design," *Pacific Symposium on Biocomputing*, vol. 5, pp. 302–313, 2000.

[9] T. Akutsu, S. Kuahara, O. Maruyama, and S. Miyano, "Identification of gene regulatory networks by strategic gene disruptions and gene overexpressions," *Proceedings of the 9th ACM-SIAM Symposium on Discrete Algorithms*, 1998.

[10] S. Liang, S. Fuhrman, and R. Somogyi, "REVEAL, a general reverse engineering algorithm for inference of genetic network architectures," *Pacific Symposium on Biocomputing*, vol. 3, pp. 18–29, 1998.

[11] B. Lemon and R. Tjian, "Orchestrated response: a symphony of transcription factors for gene control," *Genes and Development*, vol. 14, no. 20, pp. 2551–2569, October 2000.

[12] M. Merika and D. Thanos, "Enhanceosomes," *Curr Opin Genet Dev*, vol. 11, no. 2, pp. 205–208, April 2001.

[13] T. Lee, N. Rinaldi, F. Robert, D. Odom, Z. Bar-Joseph, G. Gerber, N. Hannett, C. Harbison, C. Thompson, I. Simon, J. Zeitlinger, E. Jennings, H. Murray, D. Gordon, B. Ren, J. Wyrick, J. Tagne, T. Volkert, E. Fraenkel, D. Gifford, , and R. Young, "Transcriptional regulatory networks in saccharomyces cerevisiae," *Science*, vol. 298, pp. 799–804, 2002.

[14] R. Laubenbacher and B. Pareigis, "Equivalence relations on finite dynamical systems," *Advances in Applied Mathematics*, vol. 26, pp. 237–251, 2001.

[15] R. Laubenbacher and B. Stigler, "A computational algebra approach to the reverse engineering of gene regulatory networks," *J. Theor. Biol.*, vol. 229, pp. 523–537, 2004.

[16] M. Aviñó, E. Green, and O. Moreno, "Applications of finite fields to dynamical systems and reverse engineering problems," *Proceedings of the 19th ACM Symposium on Applied Computing - SAC*, 2004.

[17] E. L. Green, "On polynomial solutions to reverse engineering problems," 2004, pre-print.

[18] O. Moreno, D. Bollman, and M. Aviñó, "Finite dynamical systems, linear automata and finite fields," *2002 WSEAS Int. Conf. on System Science Alied Mathematics & Computer Science and Power Engineering Systems*, pp. 1481–1483, 2002, also to appear in the International Journal of Computer Research.

[19] D. Bollman and E. Orozco, "Finite field models for genetic networks," 2005, preprint.

[20] D. Bollman, E. Orozco, and O. Moreno, "A parallel solution to reverse engineering genetic networks," in *Lecture Notes in Computer Science*. Springer-Verlag, 2004, vol. 3045, pp. 490–497, part III.

[21] T. Yamamoto, T. Shimura, N. Sako, Y. Yasoshima, and N. Sakai, "Neural substrates for conditioned taste aversion in the rat," *Behav. Brain Res.*, vol. 65, pp. 1231–137, 1994.

[22] Y. Yasoshima, T. Shimura, and T. Yamamoto, "Single unit responses of the amygdala after conditioned taste aversion in conscious rats," *Neuroreport*, vol. 6, pp. 2424–2428, 1995.

[23] H. Ge, R. Chiesa, and S. Peña de Ortiz, "Hzf-3 expression in the amygdala after establishment of conditioned taste aversion," *Neuroscience*, vol. 120, no. 1, pp. 1–4, 2003.

[24] Y. Robles, P. E. Vivas, H. G. Ortiz-Zuazaga, Y. Felix, and S. Peña de Ortiz, "Hippocampal gene expression profiling in spatial learning," *Neurobiology of Learning and Memory*, vol. 80, no. 1, pp. 80–95, Jul 2003.

[25] R. Chiesa, H. G. Ortiz-Zuazaga, H. Ge, and S. Peña de Ortiz, "Gene expression profiling in emotional learning with cDNA microarrays," in *40th meeting of the American Society for Cell Biology*, San Francisco, California, December 2000.

[26] R. Lamprecht, S. Hazvi, and Y. Dudai, "cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory," *J. Neurosci.*, vol. 17, pp. "8443–8450", 1997.

[27] H. Lodish, A. Berk, L. Zipursky, P. Matsudaira, D. Baltimore, and J. Darnell, *Molecular Cell Biology*. W. H. Freeman, 2000, section 20.8.

[28] T. Hubbard, D. Andrews, M. Caccamo, G. Cameron, Y. Chen, M. Clamp, L. Clarke, G. Coates, T. Cox, F. Cunningham, V. Curwen, T. Cutts, T. Down, R. Durbin, X. M. Fernandez-Suarez, J. Gilbert, M. Hammond, J. Herrero, H. Hotz, K. Howe, V. Iyer, K. Jekosch, A. Kahari, A. Kasprzyk, D. Keefe, S. Keenan, F. Kokocinsci, D. London, I. Longden, G. McVicker, C. Melsopp, P. Meidl, S. Potter, G. Proctor, M. Rae, D. Rios, M. Schuster, S. Searle, J. Severin, G. Slater, D. Smedley, J. Smith, W. Spooner, A. Stabenau, J. Stalker, R. Storey, S. Trevanion, A. Ureta-Vidal, J. Vogel, S. White, C. Woodwark, and E. Birney, "Ensembl 2005," *Nucleic Acids Res.*, vol. 33, pp. D447–D453, Jan 1 2005, database issue.

[29] J. Schug and G. C. Overton, "Tess: Transcription element search software on the www," Computational Biology and Informatics Laboratory, School of Medicine, University of Pennsylvania, Tech. Rep., 1997, technical Report CBIL-TR-1997-1001-v0.0.

[30] Rat Genome Database Web Site, "Rat genome data," Aug 2005, medical College of Wisconsin, Milwaukee, Wisconsin. World Wide Web (URL: `http://rgd.mcw.edu/`).

[31] M. Pereira-da Silva, M. Torsoni, H. Nourani, V. Augusto, C. Souza, A. Gasparetti, J. Carvalheira, G. Ventrucci, M. Marcondes, A. Cruz-Neto, M. Saad, A. Boschero, E. Carneiro, and L. Velloso, "Hypothalamic melanin-concentrating hormone is induced by cold exposure and participates in the control of energy expenditure in rats." *Endocrinology*, vol. 144, no. 11, pp. 4831–4840, Nov 2003.

[32] J. Kela, P. Salmi, R. Rimondini-Giorgini, M. Heilig, and C. Wahlestedt, "Behavioural analysis of melanin-concentrating hormone in rats: evidence for orexigenic and anxiolytic properties," *Regul. Pept.*, vol. 114, no. 2–3, pp. 109–114, Jul 15 2003.

[33] M. Varas, M. Perez, O. Ramirez, and S. de Barioglio, "Melanin concentrating hormone increase hippocampal synaptic transmission in the rat," *Peptides*, vol. 23, no. 1, pp. 151–155, Jan 2002.

[34] X. Q. Li, V. M. Verge, J. M. Johnston, and D. W. Zochodne, "CGRP peptide and regenerating sensory axons," *J. Neuropathol. Exp. Neurol.*, vol. 63, no. 10, pp. 1092–1103, Oct 2004.