[Frontiers in Bioscience 9, 104-109, January 1, 2004]

CELLULAR-RESOLUTION ACTIVITY MAPPING OF THE BRAIN USING IMMEDIATE-EARLY GENE EXPRESSION

Reza Farivar, Shahin Zangenehpour, and Avi Chaudhuri

Department of Psychology, McGill University, Montreal, Canada

TABLE OF CONTENTS

1. ABSTRACT

Immediate-Early Genes are a class of genes that are rapidly up-regulated following neural stimulation. Due to their quality as potential activity markers in the CNS, they have been used extensively in functional mapping studies. At least three genes have been popularly used, including zif268 (Egr1, NGFI, Krox-24, or ZENK), c-fos and recently, Arc. A number of techniques have been developed in applying IEG labelling for the development of functional maps, thus overcoming some of the earlier limitations of this approach. Current developments highlight the future prospects of cellular-resolution functional activity mapping of the brain.

2. INTRODUCTION

Nervous systems are in a constant dynamic state, changing connections, forming new ones and modifying their existing synaptic connections. This dynamism undoubtedly requires production of new proteins and thus a well-orchestrated genomic response. Some of these genomic responses are quite rapid and constitute a class of genes termed Immediate-Early Genes (IEG). The IEG's have diverse functions—while a large subset of them act as transcription factors that mediate delayed genomic responses, a number of them also code for structural proteins (1,13). Some IEG's are found to be down-regulated by stimulation. We have previously identified one gene, the robl/LC7-like, whose down-regulation by stimulation is detectable within 45 minutes (2,3).

The rapid appearance of IEG's makes them ideal candidates for functional activity mapping in the brain. In general, at least three different IEG's are widely used as activity markers in functional mapping. These include *c-fos*, *zif268* (Egr1, NGFI, Krox-24, or ZENK), and *Arc.* Furthermore, a number of approaches have been developed in exploiting these different activity markers, utilizing the mRNA signal, protein signal, or a combination of both. Although a number of limitations remain to be overcome, the current solutions enabling cellular-resolution functional mapping empower us to answer a number of questions that are not approachable by other functional mapping techniques such as PET or fMRI.

3. IMMEDIATE EARLY GENES

A recent micrarray study of IEG's using fetal rat tissue on an ~8000 gene-sensitive Affymatrix chip identified 95 (1.2%) genes that were up-regulated and 43 (0.5%) that were down-regulated as a result of direct activation of intracellular second-messengers (4). Berke and colleagues (5) using differential display PCR have identified a set of more than 30 genes that are up-regulated in response to stimulation of striatal dopamine D1 receptors. These studies corroborate earlier findings by Worley and colleagues who have identified a number of genes that have direct structural or physiological roles in neuronal functioning, such as Homer and Arc, in additional to various transcription factors (6,7,1). Of these IEG's,

inducible transcription factors have been most widely used as activity markers in nervous systems likely due to their earlier characterization. However, a number of recent studies have also begun to use structural IEG's such as Arc as activity markers.

3.1. Inducible Transcription Factors

Inducible Transcription Factors (ITFs) constitute a family of proteins that are products of immediate-early genes and whose function is to bind to regulatory DNA sequences and subsequently activate or repress transcription of late-response genes. These include the Fos, Jun, and EGR family of transcription factors. A comprehensive review of these transcription factors is beyond the scope of this article, and the reader is referred to (8,15). Briefly, the Fos and Jun family proteins share structural similarities in having a DNA-binding and a leucine zipper domain. The latter allows for dimerization with other leucine zipper-containing proteins. Whereas Jun proteins can form homodimers and heterodimers with proteins of the Fos and Jun families, Fos proteins can only dimerize with Jun family proteins. The different dimers of Fos and Jun proteins all bind to the same DNA regulatory element called the activator protein-a (AP-1) binding site, and the dimers are collectively called AP-1 transcription factors. The zinc finger family of transcription factors such as Egr-2 (also called Krox-20) and Egr-1 have in common a zinc finger DNA-binding domain, and both recognize similar DNA sequences. The ITF's c-Fos and Zif268 are both induced by Ca2+ influx into the cell following stimulation either through NMDA receptors or voltage-sensitive calcium channels (9) and also by numerous chemical factors (cf. 8).

Much of the effort in neuronal activity mapping with IEG's has been based on ITFs. In the 1980's, *c-fos* was shown to be present *in vivo* and related to brain activity (e.g., 10). A similar profile for zif268 was also described (e.g., 11, 12). Since then, a large number of studies have exploited the rapid induction of ITF's as markers of activity in different sensory modalities (see 13, for a review).

3.1.1. *c-fos*

c-fos was the first IEG to be characterized, and it remains as the most extensively used IEG activity marker. *c-fos* has a low basal expression level in most neural systems, making its up-regulation readily detectable. Also, the time course of *c-fos* mRNA and protein are well characterized—an increase in *c-fos* mRNA can be detected within minutes after the onset of stimulation, and peaks at about 20-60 minutes and falls to basal levels by about 2 hours. c-Fos protein levels peak at 1 to 2 hours after stimulation and returns to basal levels more slowly (14). The wide usage of *c-fos* gives it the additional benefit of reliability and testing under a wide number of diverse conditions. Furthermore, protocols and antibodies for a number of species are readily available.

One early hindrance to the usage of c-Fos protein as an activity marker was the close homologies of the fos-family proteins. Thus, true c-Fos staining could not be assured because the specific antigenic reaction was unclear. In this case, Fos-like immunoreactivity was often ascribed to ICC staining patterns. This issue has been recently resolved by the availability of antibodies that recognize epitopes specific to individual Fos members.

3.1.2 *zif268*

The *zif268* gene differs in many ways from *c-fos* in that it has a high basal level of expression in many neural structures (15, 8). *zif268* is readily up-regulated in neurons with stimuli that also induce *c-fos*. However, *zif268* has the added advantage that its down-regulation can also be studied, and this feature has been exploited in studies of sensory deprivation such as monocular deprivation (16,17). As with *c-fos*, the use of *zif268* in activity mapping also benefits from considerable staining reliability and availability of antibodies.

3.2. Functional Mapping with Inducible Structural Proteins

The Activity Regulated Cytoskeleton (Arc) factor has been studied extensively in relation to hippocampal function and memory consolidation (18-21). Arc codes for an effector protein whose RNA and protein are localized to the soma and dendrites (6). It is notable that Arc expression and localization are separable. Whereas electroconvulsive seizures are sufficient to induce Arc expression, additional high-frequency stimulation, similar to those needed for long-term potentiation, are prerequisites for dendritic Arc localization (22,23).

4. APPROACHES TO FUNCTIONAL MAPPING

The rapid induction of IEG's has been exploited in numerous mapping studies of nervous system function and dysfunction (24). The majority of IEG mapping studies have used single-epoch mapping, where the effect of a single stimulus is mapped to correlating neural regions. However, a number of recent approaches have been developed that overcome some of the limitations inherent in single-epoch mapping and enable more elaborate brain mapping studies to be conducted.

4.1. Single-Epoch Mapping

In a single-epoch mapping experiment, animals are first placed in an environment that minimizes IEG induction. For example, in our studies of visual function mapping, our animals are dark adapted for hours to days. This reduces the potential for background staining by reducing IEG responses to basal levels, thereby enhancing the detection of stimulus-induced neuronal activation. After this period of null adaptation, the animal is exposed to the stimulus of interest and, depending on whether mapping is to be conducted by mRNA or protein labelling, the tissue is obtained between 20 minutes to 2 hours. The later survival period allows for detectable protein levels to be reached, while shorter survival periods are sufficient for mRNA labelling.

Although the null adaptation period should reduce the incidence of false-positive observations, such an assumption is never firm because several extraneous factors at any point may be contributing to the observed signal.

This stimulus-transcription coupling uncertainty cannot be readily overcome by a single-epoch mapping procedure. Furthermore, in many cases it is desirable to dissociate between different cellular pools responsive to diverse stimuli and although inter-animal studies may help in this line, the approach remains limited.

4.2. Compartmental Analysis

Guzowski and colleagues (18,25,20,21) have developed a functional mapping technique based on Arc FISH, called cellular compartment analysis of temporal activity by fluorescent in-situ hybridization (catFISH) that exploits the differentiable time course of Arc mRNA localization in the nucleus versus in the cytoplasm. Specifically, at 5 min post-stimulation, Arc mRNA is detectable within the nucleus and absent in the cytoplasm, but at 30 min post-stimulation, the pattern is reversed—mRNA is detectable in the cytoplasm but not in the nucleus. The technique of Guzowski and colleagues is based on the logic advanced by Chaudhuri et al. (16), who proposed that neuronal responses to two different stimulation events could be dissociated by exploiting the differential time course of nuclear vs. cytoplasmic IEG accumulation.

The choice of *Arc* IEG as an activity marker stems from the fact that *Arc* is detectable in the nucleus within minutes (as is *zif268*), but following its cytoplasmic localization, the mRNA is distributed to the dendrites and is thus not entirely detectable, whereas *zif268* lingers in the cytoplasm for a much longer period. Another reason that Guzowski and colleagues have opted for *Arc* is the notion that *Arc* is a better marker of context effects in hippocampal neurons. However, it appears that *Arc* expression is comparable to *zif268* in its responsivity to context-induced activity in the hippocampus (19).

As described in Guzowski et al. (25), the catFISH technique has the same advantage as the dual-activity mapping technique of Chaudhuri (16) in that two different temporal events can be dissociated from one another. The specific advantage arises from the fact that various extraneous factors can be disassociated from the main stimulus under consideration, whereas in typical single-epoch mapping techniques, this uncertainty remains. One advantage of catFISH over the dual-activity mapping technique is that it only requires a single histological procedure, whereas the dual-activity mapping technique requires combined FISH and FICC. However, the major advantage of the dual-activity mapping technique over catFISH is that detection of the target products is much easier and thus possible over large spans of the cortex.

The catFISH technique, as applied thus far, has been limited to the evaluation of a few microscopic fields using a confocal microscope, and thus limited to the evaluation of only a few hundred neurons per study. Furthermore, the classification of the neurons into the different activity epochs requires careful human evaluation, making the analysis of cells labelled by this method quite time consuming. In contrast, dual activity maps can be evaluated even under low magnification (e.g., 10X) and categorization of neurons into the separate temporal epochs is simplified because by the time of tissue acquisition, genomic responses to the first stimulation schedule will have resulted in nuclear localization of the protein while the second epoch will have resulted in cytoplasmic localization of the mRNA. The separate visualization of these two genomic products using different fluorophores gives rise to a readily-discernible pattern that greatly facilitates analysis and makes it possible to conduct large-scale analyses over the entire cortex.

4.3. Mapping with Intensity Variation

An innovative extension of the single-epoch approach has been developed by Mello and colleagues (26-28). Given that any stimulus would optimally activate one set of neurons and activate others sub-optimally, how does one differentiate between these sets of neurons using IEG labelling? The approach presented by Mello and colleagues has been to categorize the variations in intensity of IEG labelling so as to have a more detailed functional map that considers not only the spatial distribution of IEG labelled cells, but also the quality of their responsiveness. With this approach, it is possible to identify regions of high, medium, and low activity in response to a stimulus, rather than simply label a region as having been "activated" by the stimulus.

Mapping with intensity variation as presented above requires a sophisticated setup, involving an automated microscope and a detection/categorization algorithm (27). The automation of this technique greatly enhances the objectivity of the approach. However, the technique is still limited by the fact that only one IEG product is traced, making within-animal comparisons difficult. Although this may not be an issue when dealing with small animals such as birds, rats or mice, the potential costs associated with such an approach when working with larger animals such as primates is substantial. Nonetheless, the consideration of intensity of labelling is an important one for the accurate estimation of cellular-resolution functional maps.

4.4. Dual Activity Mapping

We have previously developed a molecular mapping technique that allows us to identify separate pools of neurons that are responsive to two different stimulus schedules (16,29). Our approach is based on the differential induction time course of an IEG mRNA and protein signal. Transcription factors such as zif268 reach detectable mRNA levels within 30 minutes following stimulation, whereas the nuclear protein signal reaches its peak within 2 hours. The dual-activity mapping approach exploits this differential time course by exposing an animal to two different stimulation events that are timed to take advantage of the different inducible responses (14). In our studies, animals experience one period of stimulation for 30-45 minutes followed by a period of null adaptation, which is then followed by an additional 30 minutes of stimulation. Neurons responsive to stimuli in the first event will have high levels of the IEG proteins localized to their nucleus, whereas neurons responsive to the stimuli in the second event will have high levels of the mRNA localized in their

cytoplasm. Finally, neurons that were responsive to both stimulation events or that were active due to extraneous factors will have both genomic responses present. We employ fluorescence in-situ hybridization (FISH) and fluorescence immunocytochemisty (FICC) in conjunction with tyramide signal amplification (TSA), making double- and single-labelled neurons observable even under low magnification.

We have so far validated this approach under different conditions. Originally, we showed that ocular dominance columns for both eyes can be visualized with this technique (16). Since this discovery, we have applied this approach to the analysis of multisensory neurons in the neocortex and superior colliculus of the rat (29), where we were able to observe a pool of neurons responsive to auditory or visual information, and also neurons that were double-labelled—representing bimodal neurons responsive to both auditory and visual stimulation. We are currently extending this approach to map higher-level visual cognitive functions such as face perception in primates (30).

5. STRENGTHS AND CAVEATS OF MOLECULAR MAPPING

Molecular mapping using IEG markers has a number of advantages and disadvantages over other functional mapping techniques:

1. *Rapid induction.* IEG mRNA up-regulation can be detected within minutes of stimulation, making it very sensitive to short stimulation schedules. The protein signal can take considerably longer to develop, but even this slower signal can develop well within 60 to 90 minutes. The low stimulation requirement means that IEG mapping can be conducted under many different behavioural situations.

2. *Cellular resolution mapping.* Other than electrophysiological recordings, no other technique can afford activity detection with such precise localization. However, molecular mapping techniques allow for single-cell resolution over large spans of cortex, something that is not possible with electrophysiology.

3. *Application with other histological techniques.* Sections labelled for IEG products can be post-treated with probes for other proteins such as receptors or neurofilaments, or filled using Lucifer Yellow (31,32) allowing for precise neuroanatomical and neurochemical analysis. Furthermore, the approach can be readily combined with tract-tracing procedures for a more comprehensive neuroanatomical study.

4. *Large-scale analysis with cellular-resolution.* Molecular mapping techniques as described above allow for analysis of large areas of the CNS while maintaining cellular-level resolution.

Flexible behavioural schedules. Given that all analysis is conducted post-mortem, a flexible behavioural schedule is available to the experimenter. Unlike PET, fMRI, or electrophysiological techniques, there are no preparatory procedures that need to be conducted for a molecular mapping study. Instead, the animals are enabled to behave more naturally in potentially unrestricted environments.

However, a number of caveats hinder the general application of molecular mapping techniques. These include:

1. *Poor temporal resolution.* IEG induction has a poor dynamic quality and is therefore not sensitive to rapid temporal changes. As a result, small changes in behaviour or stimulation can go undetected.

2. *Quantitative analysis.* In general, quantitative analysis of products using histological techniques has been limited, with few exceptions (26,27). Some quantification is possible by means of cell counts, but this is not always informative.

3. *Expression specificity.* Not all neurons express all IEG's. For example, zif268 expressed primarily within a subset of excitatory neurons (33). Thus the absence of IEG labelling in some cells cannot be readily taken as the absence of activity in those neurons.

False-positives. Neural activity does not have an exclusive association with IEG induction, as discussed earlier. In addition to neural activity, other factors such as endocrine stimulation can sometimes be implicated in IEG induction that may compromise the information obtained from the staining patterns.

6. FUTURE DIRECTIONS

The future holds considerable promise for functional mapping with currently available and newly discovered IEG products. A number of new techniques have been developed to overcome earlier limitations by considering time course of expression (25), intensity of labelling (25), and multiple genomic responses (16,29). The application of these techniques will allow for a high-precision level of analysis, since neural correlates of multiple behaviours can be dissociated within the same animal and that variations in activity can be accounted for by variations in labelling intensity. However, in comparison to other mapping techniques much remains to be achieved.

First, molecular mapping methods are typically limited to a few sections and often few microscopic fields within those sections. This can sometimes be a hindrance in interpreting the data in terms of a global perspective. This limitation arises because a number of variables are introduced in large-scale analyses. For example, in attempting to conduct molecular mapping across the cortex, one should be considering spatial distribution of IEG expressing cells and possibly even the intensity of their labelling. Yet even the spatial distribution of labelled cells varies, making it unclear what an "activated" region really is and how activity in that region is organized. This

highlights our lack of an analytic technique in describing and defining the variations in the spatial distribution of activated neurons.

The above issue illustrates yet another problem--in order to evaluate spatial distributions over large spans of the brain, one would need large images of those areas while maintaining cellular-resolution mapping. Fortunately, automated microscopes and automation kits are becoming more and more affordable, thus potentially solving this issue and enabling researchers to evaluate large spans of the brain by constructing a whole-tissue image from the individual microscopic fields.

The availability of whole-tissue images would overcome another limitation of current molecular mapping efforts. Whereas with PET and fMRI one can navigate a volumetric model of the brain, this is not readily possible with molecular mapping methods. This is largely due to the fact that whole-tissue images are rarely constructed for these purposes, but also because the digital size of such a volumetric file with cellular-resolution would be unmanageable even for some of the higher-end workstations. A potential future development in this area would be data reduction techniques that would make such volumetric visualization possible. One potential approach is to conduct automated cell counts using readily available algorithms, and to then construct the volumetric model from simple cell positions, discarding image information thereby reducing the digital size of the model.

Volumetric modelling of molecular mapping studies would offer tremendous benefits to researchers. Whereas comparisons across individuals are painless with PET and fMRI, this comfort is not afforded to functional molecular mapping efforts. Advances in volumetric reconstruction of histological sections labelled for IEG's would empower the combination of this cellular-resolution mapping technique with other mapping efforts such as electrophysiological recordings or even fMRI and PET in animals.

Additional technical developments still needed concern the accommodation of multiple-stimulus mapping techniques. Current approaches are limited to the evaluation of two stimulation events. This is not entirely satisfactory for more sophisticated mapping studies. One immediate possibility will be to combine a dual-activity mapping approach with a third or even fourth marker. A potential approach may be to utilize some of the later response genes (4), such as TOE1 (Target of EGR-1; 34). It may thus be feasible to combine the mRNA and protein expression of a factor such as TOE1 as two additional temporal markers, although factors such as suppression of IEG response by these late-response genes ought to be carefully considered (35). And finally, it seems feasible to combine the rapid up-regulation of a gene such as Arc with our technique. As noted previously, Arc mRNA is detectable within the nucleus within minutes (18), and this quality may enable us to readily combine it with the current dual-activity technique to label a third-stimulation event.

7. CONCLUSION

The application of IEG labelling techniques for functional activity mapping provides a powerful tool with for the investigation of neural function, organization, and anatomy. There have been numerous innovative developments over the past few years, as discussed in this review. It is now apparent that functional mapping techniques utilizing IEG products have secured their place in the repertoire of functional imaging techniques in the neurosciences.

8. REFERENCES

1. Lanahan A, P. Worley: Immediate-early genes and synaptic function. *Neurobiol Learn Mem* 70:37-43. (1998)
2. Chaudhuri A, S. Zangenehpour, F. Rahbar-Dehgan, F.Ye: Molecular maps of neural activity and quiescence. *Acta Neurobiol Exp* (Warsz) 60:403-410. (2000)
3. Ye F, S. Zangenehpour, A. Chaudhuri: Light-induced down-regulation of the rat class 1 dynein-associated protein robl/LC7-like gene in visual cortex. *J Biol Chem* 275:27172-27176. (2000)
4. Mayer P, S. Ammon, H. Braun, H. Tischmeyer, U. Riechert, E. Kahl, V. Hollt: Gene expression profile after intense second messenger activation in cortical primary neurones. *J Neurochem* 82:1077-1086. (2002)
5. Berke JD, RF Paletzki, GJ Aronson, SE Hyman, CR Gerfen: A complex program of striatal gene expression induced by dopaminergic stimulation. *J Neurosci* 18:5301-5310. (1998)
6. Lyford GL, K. Yamagata, W.E. Kaufmann, C.A. Barnes, L.K. Sanders, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, A.A. Lanahan, P.F. Worley: Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* 14:433-445. (1995)
7. Brakeman PR, A.A. Lanahan, R. O'Brien, K. Roche, C.A. Barnes, R.L. Huganir, P.F. Worley: Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* 386:284-288. (1997)
8. Herdegen T, J.D. Leah: Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Res Brain Res Rev* 28:370-490. (1998)
9. Ghosh A, D.D. Ginty, H. Bading, M.E. Greenberg: Calcium regulation of gene expression in neuronal cells. J *Neurobiol* 25:294-303. (1994)
10. Sagar SM, F. R. Sharp, T. Curran: Expression of c-fos protein in brain: metabolic mapping at the cellular level. *Science* 240:1328-1331. (1988)
11. Herdegen T, T. Walker, J.D. Leah, R. Bravo, M. Zimmermann: The KROX-24 protein, a new transcription regulating factor: expression in the rat central nervous system following afferent somatosensory stimulation. *Neurosci Lett* 120:21-24. (1990)
12. Worley PF, B.A. Christy, Y. Nakabeppu, R.V. Bhat, A. J. Cole, J. M. Baraban: Constitutive expression of zif268 in neocortex is regulated by synaptic activity. *Proc Natl Acad Sci U S A* 88:5106-5110. (1991)

13. Chaudhuri A, S. Zangenehpour: Molecular activity maps of sensory function. In: Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction. Eds: Kaczmarek L, Robertson HA. Amsterdam: Elsevier Science B.V. (2002)
14. Zangenehpour S, A. Chaudhuri: Differential induction and decay curves of c-fos and zif268 revealed through dual activity maps. *Brain Res Mol Brain Res* 109:221-225. (2002)
15. Kaczmarek L, A. Chaudhuri: Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for functional mapping and neural plasticity. *Brain Res Brain Res Rev* 23:237-256. (1997)
16. Chaudhuri A, J. Nissanov, S. Larocque, L. Rioux: Dual activity maps in primate visual cortex produced by different temporal patterns of zif268 mRNA and protein expression. *Proc Natl Acad Sci U S A* 94:2671-2675. (1997)
17. Kaczmarek L, S. Zangenehpour, A. Chaudhuri: Sensory regulation of immediate-early genes c-fos and zif268 in monkey visual cortex at birth and throughout the critical period. *Cereb Cortex* 9:179-187. (1999)
18. Guzowski JF, B. L. McNaughton, C. A. Barnes, P.F. Worley: Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. *Nat Neurosci* 2:1120-1124. (1999)
19. Guzowski JF, G.L. Lyford, G.D. Stevenson, F. P. Houston, J. L. McGaugh, P. F. Worley, C. A. Barnes: Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J Neurosci* 20:3993-4001. (2000)
20. Guzowski JF, B. Setlow, E. K. Wagner, J. L. McGaugh: Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. *J Neurosci* 21:5089-5098. (2001)
21. Guzowski JF: Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. *Hippocampus* 12:86-104. (2002)
22. Steward O, C. S. Wallace, G. L. Lyford, P. F. Worley: Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. *Neuron* 21:741-751. (1998)
23. Wallace CS, G. L. Lyford, P. F. Worley, O. Steward: Differential intracellular sorting of immediate early gene mRNAs depends on signals in the mRNA sequence. *J Neurosci* 18:26-35. (1998)
24. Kaczmarek L, H. A. Robertson: Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction. Amsterdam: Elsevier Science B.V. (2002)
25. Guzowski JF, B. L. McNaughton, C. A. Barnes, P. F. Worley: Imaging neural activity with temporal and cellular resolution using FISH. *Curr Opin Neurobiol* 11:579-584. (2001)
26. Ribeiro S, G. A. Cecchi, M. O. Magnasco, C. V. Mello: Toward a song code: evidence for a syllabic representation in the canary brain. *Neuron* 21:359-371. (1998)
27. Cecchi GA, S. Ribeiro, C. V. Mello, M. O. Magnasco: An automated system for the mapping and quantitative analysis of immunocytochemistry of an inducible nuclear protein. *J Neurosci Methods* 87:147-158. (1999)
28. Mello CV: Immediate-early gene (IEG) expression mapping of vocal communication areas in the avian brain. In: Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction. Eds: Kaczmarek L, Robertson, H.A, 59-101. Amsterdam: Elsevier Science B.V. (2002)
29. Zangenehpour S, A. Chaudhuri: Neural activity profiles of the neocortex and superior colliculus after bimodal sensory stimulation. *Cereb Cortex* 11:924-935. (2001)
30. Zangenehpour S, A. Chaudhur: Focal clustering of face-selective neurons in monkey temporal lobe. In: Society for Neuroscience. Orlando. (2002)
31. Wegerhoff R, O. Breidbach: Intracellular dye injection of previously immunolabeled insect neurons in fixed brain slices. *J Neurosci Methods* 53:87-93. (1994)
32. Pace CJ, D. G. Tieman, S. B. Tieman: Intracellular injection in fixed slices: obtaining complete dendritic arbors of large cells. *J Neurosci Methods* 119:23-30. (2002)
33. Chaudhuri A, J. A. Matsubara, M. S. Cynader: Neuronal activity in primate visual cortex assessed by immunostaining for the transcription factor Zif268. *Vis Neurosci* 12:35-50. (1995)
34. De Belle I, J. X. Wu, S. Sperandio, D. Mercola, E. D. Adamson: In vivo cloning and characterization of a new growth suppressor protein TOE1 as a direct target of Egr1. *J Biol Chem*. (2003)
35. De Cesare D, P. Sassone-Corsi: Transcriptional regulation by cyclic AMP-responsive factors. *Prog Nucleic Acid Res Mol Biol* 64:343-369. (2000)

Key Words: Immediate-Early Genes, Molecular Mapping, Neural Activity, Egr-1, Zif268, Arc, gene expression, dual activity mapping. c-fos, IEG

Send correspondence to: Reza Farivar, Department of Psychology, McGill University, Montreal, Canada, H3G 1B6. Tel: (514) 398-6151, Fax: (514) 398-3255 E-mail: reza.farivar@mail.mcgill.ca