STANDARDIZED ANALYSIS OF c-fos mRNA EXPRESSION PATTERN IN RAT BRAIN AUTORADIOGRAPHY USING FREEWARE IMAGING SOFTWARE

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Activation of the proto-oncogene *c-fos* in the brain was described initially two decades ago and several studies have shown that different stimuli can induce *c-fos* expression.
c-fos in situ hybridization is commonly used in pharmacological studies to map rat or mouse brain areas involved in the effects of different treatments.
c-fos expression is relatively low in basal conditions, therefore autoradiography of c-fos hybridized brain does not show a strong signal. As a consequence, the identification of brain areas activated after an experimental condition can be often very difficult and usually relies upon the anatomical skills of the experimenter. The identification step is usually followed by the quantification procedure consisting of measuring the optical density of a sampling area of standard size for each hybridized brain region.
Here we propose a novel method for standardized identification and analysis of autoradiography *c-fos* mRNA expression that does not require sampling areas but allows to evaluate the optical density of the whole brain region hybridized.
This method is based on the use the freeware software IMAGEJ. In our experiments c-fos hybridized slides from rat brains were exposed, together with [14C]-microscale standards, to X-ray film for 3 days before developing. Autoradiograms were captured digitally at high resolution and divided into single slice images for quantification. A calibration curve was obtained measuring the optical density of the [14C]-microscale standards. Each brain slice was superimposed with a drawing obtained from the Paxinos and Watson atlas (1998). In particular, from the CD rom of the atlas a proper plate was chosen that could correspond to the brain slice to analyze. The *pdf* file of such plate was transformed into a *tiff* file calculating the proper pixel to mm ratio that reproduced the same resolution and the same size of the brain slice. The *tiff* file containing the drawing from the atlas was then opened with the IMAGEJ software and superimposed on the brain slice to analyze. In this way the brain area of interest could be identified and selected automatically using the "wand tool" of the software, able to detect the area contour from the atlas drawing. The optical density results obtained with this method were compared to those obtained following a sampling area procedure and no statistically significant difference was observed.