Expression of c-Fos in response to stressogenic stimuli in the amygdala of old vs. young rats – a preliminary study

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Abstract. During aging many of the brain functions became deteriorated or altered. One of the most important age related changes is an increase of anxiety level, reported both in humans and in animals. Our study was intended to compare c-fos gene expression in amygdala, the key structure in anxiety/fear regulation, in old (24 months) and young (4 months) rats exposed to various behavioral stimulations. There were no differences between age groups in basal c-Fos expression. After social encounter c-Fos expression level in amygdala increased significantly, but still remained independent on age. Significant differences between both groups appeared after open field test and immobilization test. Contrary to the findings on young adults indicating the correlation between increased anxiety level and higher c-Fos expression, old rats showed increased anxiety together with significantly lower c-Fos expression.

Key words: age related, aging, anxiety, rats, c-Fos, amygdala
Aging is a multifactorial process which causes not only deterioration of cognitive performance, but also induces various alterations in emotional behavior. Several studies in recent years have reported an age related increase of anxiety level in rodents (Blokland and Raaijmakers 1993, Boguszewski and Zagrodzka 2002, Frussa-Filho et al. 1992, Lamberty and Gower 1992, 1993, Li et al. 1995, Miyagawa et al. 1998). It is well known that new or anxiogenic stimuli lead to activation of so-called brain defense system, i.e., integrated neuronal circuitry that detects threatening or stressogenic stimuli and organizes response to them. Within this circuitry amygdala is considered as a key structure in processing the emotional information (Aggleton 1992, LeDoux 1998).

One of the methods successfully used to label activation of different brain structures in response to various experimental manipulations is assessment of the expression of transcription factors (Kaczmarek 2002). With this approach a correlation between anxiety and c-Fos induction in brain areas implicated in anxiety regulation has been demonstrated in young adult rats (Duncan et al. 1996, Abraham and Kovacs 2000).

The aim of our preliminary study was to investigate the possible differences in c-Fos expression in amygdala of young in comparison with old rats exposed to various anxiogenic stimuli. The induction and analysis of c-Fos expression was a final part of more complex experiment designed to investigate age-related behavioral and biochemical changes in emotional and social reactivity in rats.

Each particular stage of this experiment was performed on young – aged 4 months (YA), and old – aged 24 months (OA) male Wistar rats of the same strain, bred in the licensed animal husbandry of the Institute of Occupational Medicine in Lodz. The project was approved by the Ethic Committee of the Nencki Institute. Before the proper experiment the rats were subjected to a series of sensorimotor tasks designed to assess muscle strength and coordination, equilibrium and orienting reactions in order to assure the homogeneity of the group, i.e., animals with large physical impairments were eliminated from the further study.

All animals (except control group for c-Fos, see below) were tested in a set of standard laboratory tests that examine spontaneous behavior and include emotional as well as motor components, i.e., elevated plus maze test (EPM), open field with illuminated center test (OF) and social interaction test (SI). Each test was performed only once according to earlier established schedule. Data were collected and analyzed with video-based EthoVision system (Noldus, The Netherlands). (For details of the method, see Boguszewski and Zagrodzka 2002).

The behavioral results evaluated with principal component analysis (PCA) showed in old animals when compared to young individuals, increased level of anxiety as well as decreased motor activity in OF and EPM. PCA proved that these factors are independent, thus represent distinct age-related processes. As to social interactions our results demonstrated that the pattern of social encounters was totally distinct in old animals compared to young ones, while number and time spent on social interaction did not show age related differences (Boguszewski and Zagrodzka 2002).

Rats destined for immunocytochemical analysis of c-Fos expression were divided into four groups: (1) control group (CO) taken directly from home cage; (2) animals subjected to immunocytochemistry after social encounter (SI); (3) animals subjected to c-Fos analysis after open field with illuminated center (OF); (4) animals subjected to c-Fos staining after immobilization procedure (I) consisting of a 15 minutes stay in ventilated Plexiglas transparent tube. c-Fos analysis was performed 90 min after the beginning of behavioral procedure – animals were anesthetized with an overdose of chloral hydrate and perfused intracardially. Immunocytochemistry on amygdala sections was performed according to the standard procedure (Kaminska et al. 1996). In this preliminary study the divisions of amygdala have not been taken into consideration. c-Fos immunoreactivities were measured in the whole structure and expressed as number of c-Fos immunopositive nuclei per amygdala. Measurement was done by image analysis system (analySIS Olympus).

The level of residual c-Fos expression in amygdala in control animals was similar in young and old rats (Fig. 1). This is in agreement with earlier studies, where not only c-Fos but also others transcription factors were investigated (Desjardins et al. 1997, Smith et al. 2001). Each behavioral manipulation in our experiment induced an increase of c-Fos expression in young, as well as in old animals. In both groups the level of c-Fos expression was statistically higher after open field test than after social interactions (Fig. 1). Taking into account findings that indicate direct correlation between anxiety and c-Fos induction in the brain
Fig. 1. c-Fos expression in amygdala. The data represent the average number of c-Fos immunostained cells per whole structure. Values are mean ± SEM, n=2 (for each situations 2 rats from group and 4 slices per rat). Statistical significance: ANOVA followed by LSD Duncan test; (*) P<0.05; (**) P<0.01.

defense system (Abraham and Kovacs 2000, Duncan et al. 1996), it might be suggested that social interactions create less stressful situation (especially because our animals were socially housed) than exposure to novelty and bright light in open field arena. Significant differences between groups appeared only after open field test and immobilization (Fig. 1). Contrary to our expectations based on the reports mentioned above (Abraham and Kovacs 2000, Duncan et al. 1996), c-Fos expression was significantly lower in old rats although they demonstrated increased anxiety in OF and EPM. Similar results, i.e., lower c-Fos expression in old rats compared to young individuals were recently described after social encounters by Salchner and coauthors (2004). Old animals showed decreased number of social contacts, which according to authors reflects increased anxiety. It should be mentioned however that this study was performed on Sprague-Dawley rats and with a different social interaction procedure.

Lower c-Fos expression in old animals might be caused by reduced ability to activate neurons as a result of aging of brain tissue. It should be pointed out that lower induction of c-Fos in old rodents after cognitive tasks engaging learning and memory was reported by Nagahara and Handa (1997) and Touzani and coauthors (2003).

Age related differences in the dynamics of c-Fos expression might serve as another explanation to be taken into account. Studies of dynamic curve of mRNA level in function of time after pharmacological seizure’s induction demonstrated that in young rats maximum concentration was reached after 1 hour and returned to basal level after 2 hours, whereas in old animal maximum was reached after 3 hours and returned to basal level after 15 hours (Retchkiman et al. 1996, Wagner et al. 2000). In the present study lower c-Fos induction in old rats was observed also after immobilization. Immobilization as an extremely stressful procedure is rather expected to evoke maximum expression of the gene.

Taken together our results confirmed that c-Fos expression might be used as a valuable molecular indicator of animal response to various stressogenic stimuli. Present data however do not support hypothesis of direct correlation of fear level and transcription factors expression. Further studies with additional calibration of c-Fos expression curves in different age groups are needed to clarify the role of age factor in this correlation since there are only two reports (including the present one) devoted to the relations age-anxiety – c-Fos expression.

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