PREFRONTAL CORTEX ACTIVITY PATTERNS DURING TASTE NEOPHOBIA HABITUATION IN ADULT AND AGED RATS

Authors: Expósito A.N. [1]*, Morillas E. [1], Gómez-Chacón B. [1], Gallo M. [1]

[1] Dept. of Psychobiology. Institute of Neurosciences. Center for Biomedical Research (CIBM). University of Granada. Spain.

*Corresponding Author:

Alejandro Navarro Expósito
Institute of Neurosciences
University of Granada
Center for Biomedical Research (CIBM)
Avda del Conocimiento, s/n
Parque Tecnológico de la Salud (PTS)
18016- Armilla
Granada (Spain)
Email: anexposito@ugr.es

Abstract

Age-related memory decline has been associated with changes in the medial prefrontal cortex (mPFC) function. In order to explore the role of mPFC in taste recognition memory, we have assessed mPFC c-Fos immunoreactivity in adult (5-month-old) and aged (24-month-old) male Wistar rats during the first (Novel), second (Familiar I), and sixth (Familiar II) exposure to a cider vinegar solution. Adult brains showed higher c-Fos expression in the ventral but not the dorsal region of mPFC during the second taste exposure. Interestingly, old brains exhibited an altered activity pattern selectively in the dorsal peduncular cortex (DP) which can be associated with a delayed attenuation of vinegar neophobia in this group. These results support the involvement of this area in the formation of safe taste memory. Further research is needed for understanding the role of DP in taste recognition memory and the impact of aging on it.

Keywords: flavor, recognition memory, infralimbic, prelimbic, dorsal peduncular, aging

Age-related cognitive decline is evident in a variety of memory impairments that involve the prefrontal cortex, a vulnerable brain region [1]. Among them, recognition memory has been proposed as the purest measure of age-related memory deficit in humans [2]. The ability to recognize previously encountered stimuli as familiar can be studied in rodents through different sensory modalities. Aged mice [3] and rats [1,4] exhibit impairments in visual recognition memory tests. These impairments have been associated with altered brain activation in areas such as the perirhinal cortex and the hippocampus [3], as well as a reduced volume of the striatum, the hippocampus, and the medial prefrontal cortex (mPFC) [1]. Meanwhile, taste recognition memory is relatively well preserved during aging. Taste neophobia which consists in the reduced intake of novel tastes is evident in aged rats [5,6]. The attenuation of taste neophobia (AN) upon repeated taste exposures without consequences is delayed in aged rats [7,8]. This age-related delay in AN has been associated with altered activity patterns in the perirhinal cortex [7], the piriform cortex [9] and the nucleus accumbens [10] using c-Fos expression as an index of neural activity. While aged rats exhibit higher activity than adult rats in the perirhinal cortex [7] and the nucleus accumbens [10] shell during drinking a familiar vinegar solution after six exposures, an age-related overall increased activity in the piriform cortex not related to flavor familiarity has been reported [9].

Previous evidence has pointed out the interaction between the nucleus accumbens and mPFC during taste familiarization in adult rats. It has been shown that depletion of mPFC dopamine (DA) by 6-OHDA lesions prevented the reduced accumbens DA response to a familiar palatable chocolate taste solution thus maintaining the DA response to taste novelty [11]. As far as we know, to date this was the only study suggesting the potential involvement of mPFC in taste familiarization. Nonetheless,

there is plenty of evidence showing the implication of mPFC in other taste memory tasks such as conditioned taste aversion (CTA) [12–15] and flavor preference learning [16,17]. Furthermore, mPFC seems to have a particular role in the extinction of learned responses including CTA [15,18,19]. In fact, this has been proven through immunohistochemical c-Fos determination, showing that extinction of a learned taste aversion is associated with increased mPFC activity [20]. Given that CTA extinction involves a shift of the taste's hedonic value from aversive to safe, it can be hypothesized a similar involvement of mPFC in the hedonic shift taken place during AN.

A plethora of studies in different species, including rodents, have reported that the prefrontal cortex is especially vulnerable to the effects of aging [21]. Therefore, it seems valuable to extend our previous work on AN-related brain activity [7,9,10] to the mPFC. The rat's mPFC is a heterogeneous brain region that can be subdivided into different subdomains according to a variety of criteria (Fig 1a). Based on cytoarchitectonic features, mPFC can be divided into anterior cingulate (ACC), prelimbic (PrL), infralimbic (IL), and dorsal peduncular (DP) cortices [22,23]. According to anatomical criteria and connectivity with other brain areas, it has been suggested a subdivision into a dorsal component (dmPFC) that includes ACC and the dorsal region of PrL, versus a ventral component (vmPFC) that includes the ventral region of PrL, IL, and DP [24]. This latter component seems to be involved in flexible shifting to new strategies in spatial learning [24], which is in accordance with the reported involvement of IL in CTA extinction [15,19].

In order to explore the effects of aging on mPFC activity during taste neophobia and its attenuation, we assessed c-Fos protein expression as an index of neural activity in adult and aged rats during the first (Novel), the second (Familiar I), and the sixth (Familiar II) exposure to a vinegar solution. This technique has been extensively used for mapping

the brain areas activated during the exposure to novel and familiar tastes because c-fos is an immediate early gene expressed transiently and rapidly when neurons are active thus reflecting recent activity more precisely than other markers of neural activity. We compared c-Fos expression using two different classifications for mPFC regions: a dorso-ventral division including the two components dmPFC and vmPFC; and a second more conventional division including ACC, PrL, IF, DP (Fig. 1a).

As it has been previously reported [9], 21 adult (5-month-old) and 24 aged (24-month-old) male Wistar rats were housed individually and maintained on a 12 h light/dark cycle (light from 08:00 to 20:00h). Food was available ad libitum but they were subjected to a water deprivation schedule with two daily drinking sessions (15 minutes during the morning and 20 minutes during the afternoon). After stabilizing the water drinking baseline, the animals had access to a non-palatable cider vinegar solution (3%) during the morning drinking sessions for six consecutive days. It has previously reported no differences in acid taste preferences between adult and aged [25]. Intake (ml) was recorded by weighing the drinking tubes before and after each drinking session. According to the experimental day in which they were euthanized to perform immunohistochemistry of the protein c-Fos, the rats were assigned to the following groups: adult (n = 7) and aged (n = 8) rats euthanized on day 1 (Novel group); adult (n = 7) and aged (n = 8) rats euthanized on day 2 (Familiar I); and adult (n = 7) and aged (n = 8) rats euthanized on day 6 (Familiar II). Euthanasia took place 90 minutes after the corresponding drinking session. The experimental procedure was approved by the University of Granada Ethics Committee for Animal Research and Junta de Andalucía (17-02-15-195).

The immunohistochemical procedure has been described elsewhere [7,9,10]. In brief, under deep anaesthesia the rats were transcardially perfused with 0.9% saline followed

by 4% paraformaldehyde, the brains were removed and coronal sections approximately between +3.24 mm and +2.76 mm relative to bregma were cut at 20 µm using a cryostat (Leica CM1900). Tissue sections were then rinsed in phosphate-buffered saline (PBS 0.01M, pH 7.4), incubated in 3% goat serum and 0.4% Triton X-100 in PBS for 30 min. The slices were transferred to a c-Fos primary antibody (Biotinylated goat anti-rabbit IgG, 1:500; Calbiochem) for 120 min at room temperature. Primary and secondary antibody solutions were mixed in a solution of 2% normal goat serum, 0.4% Triton X-100, and PBS. The sections were rinsed again and processed using the ABC kit (Vector Laboratories, Burlingame, CA). The reaction was visualized using the peroxidase substrate kit (DAB) (Vector Laboratories, Burlingame, CA). Finally, they were rinsed, mounted on gelatine-subbed slides, rehydrated with ethanol and xylenes and finally they were cover-slipped.

In order to quantify c-Fos positive cells, 14 digital images per hemisphere from two different sections were captured in each brain using a light microscope (Olympus BX41) at 40x magnification following a rostro-caudal division. As shown in Figure 1a, the total number of images taken in each subdivision and hemisphere was: ACC: 6, PrL: 10, IL: 6 and DP: 6. When considering the dorso-ventral division, this means that we took 12 images in dmPFC and 16 in vmPFC. The number of Fos-positive cells was counted automatically using the Image J software (National Institute of Mental Health). For each image's threshold objects having specific area (25-135) and circularity (0.25-1.00) values matching those of c-Fos positive nuclei were automatically counted by the software. Beforehand, in order to equalize all images and cancel out the background noise, they were converted into 8-bit type image and the background was lightened (50.0 pixels). Mean values were calculated for each brain area.

The behavioural results corresponding to the Familiar II groups are available elsewhere [9]. In brief, both adult and aged groups exhibited vinegar but the aged groups showed a delayed AN requiring one more vinegar exposure in order to reach the same amount of vinegar intake than that of Day 6. There were no differences between adult and aged groups in water intake during the baseline or in vinegar consumption during the drinking sessions prior to euthanasia: novel [$F(1, 13) = .36$; $p = .55$], familiar I [$F(1, 13) = .4.21$; $p = .06$] and familiar II [$F(1, 13) = .043$; $p = .83$] (Table 1).

The mean (±SEM) number of c-Fos positive cells of both adult and aged rats under the three taste familiarity conditions (Novel, Familiar I, and Familiar II) is shown in Figure 2. Independent 2x3 bifactorial ANOVA (age x familiarity) analyses were performed for each area using two different classifications: dmPFC and vmPFC (Figure 2a); and ACC, PrL, IF, DP (Figure 2b).

Regarding dmPFC and vmPFC, after analyzing the homogeneity of variance (Levene 6, 5607 = 0.582, $p = .74$), two-way ANOVAs indicated a significant interaction age x familiarity in vmPFC [$F(2,34)=4.850$; $p<.05$, $\eta^2 = .22$] but not in dmPFC. Analysis of the interaction revealed a significant effect of familiarity [$F(2,17)=34.144$; $p<.01$, $\eta^2 = .82$] in adult rats. Post-hoc Bonferroni tests showed a higher number of c-Fos positive cells in the Familiar I group than in the Novel ($p< 0.01$) and the Familiar II ($p < 0.01$), while these latter two groups did not differ in c-Fos positive cells. A one-way ANOVA of the factor familiarity in aged groups did not reach. However, old rats exhibited higher c-Fos immunoreactivity than adult rats during drinking the novel vinegar solution for the first time ($p < .05$).

For to the more conventional division of mPFC in ACC, PrL, IL, and DP, after analyzing the homogeneity of variance (Levene F (50, 1924) = 1.924, $p = .12$), we

performed independent 2 x 3 (age x familiarity) ANOVAs for each area. No significant effects of the main factor or interaction were found in ACC. In PrL the analysis yielded only a significant effect of the main factor familiarity [$F(2,34)=5.158$; $p<0.05$, $\eta^2 = .22$]. Bonferroni post-hoc test revealed a higher number of c-Fos positive cells in the Familiar I group in comparison with the Novel ($p<0.05$) but not the Familiar II group. Similar results were found in IL, where only the main factor familiarity was found significant [$F(2,34)=5.377$; $p<0.01$, $\eta^2 = .24$]. Nonetheless, in this case the Bonferroni test showed a higher number of c-Fos positive cells in the Familiar I group than both in the Novel ($p < 0.05$) and Familiar II ($p < 0.05$) groups. Regarding DP, in addition to a significant main effect of familiarity [$F(2,34)=6.064$; $p< 0.01$, $\eta^2 = .33$], the interaction age x familiarity was also found significant [$F(2,34)=6.064$; $p<0.01$, $\eta^2 = .26$]. Analysis of the interaction revealed significant main effects of the familiarity factor in both adult [$F(2,15)=8.524$; $p<0.01$, $\eta^2 = .53$] and aged rats [$F(2,19)=6.306$; $p<0.01$, $\eta^2 = .39$]. But interestingly enough, the Bonferroni-corrected test revealed a different pattern of c-Fos expression for each age group. Adult rats showed a similar pattern to the one found in other mPFC subdivisions, exhibiting a higher number of c-Fos positive cells in the Familiar I group than both the Novel ($p<0.01$) and the Familiar II ($p<0.05$) groups. However, aged rats exhibited a higher number of c-Fos positive cells in the Novel group than in the Familiar II ($p<0.01$), and a tendency to a higher number of c-Fos positive cells in the Familiar I group in comparison with the familiar II ($p=.06$). Comparisons between aged and adult groups yielded significant differences in all the familiarity conditions. Aged rats exhibited higher c-Fos expression than adult rats in the Novel condition ($p=0.05$) but lower in Familiar I ($p=0.05$) and Familiar II ($p=0.05$) conditions, thus indicating a different pattern of activity in DP for old and adult rats.

Our results demonstrate for the first time changes in mPFC activity associated with taste recognition memory which are altered by aging. These changes cannot be attributed to age-related differences in taste volume consumption or other non-specific motivational changes because no differences between adult and aged groups were found in water or vinegar intake. The use of two different classifications of mPFC subdivision allows us to draw accurate conclusions about the role of different mPFC regions. Using a dorso-ventral division, our data indicate that ventral but not dorsal mPFC increases c-Fos expression during the second taste exposure in comparison with the first and the sixth exposures in adult rats. This supports the potential involvement of vmPFC in the formation of safe taste memory but not in the neophobic response nor in taste memory long-term maintenance. This finding is confirmed and extended by a more precise analysis using the second classification in which we included PrL and IL, as the most frequent regions used in memory studies, and we add ACC and DP. The results confirmed the same activity pattern in PrL, IL and DP. A similar activity pattern in adult rats has been previously reported in the nucleus accumbens shell [10] which is consistent with the proposed mPFC-accumbens shell interaction relevant for familiarization [11]. As these authors propose it is conceivable that vmPFC codes for the generic motivational value of the taste which in the present experiment is increased during AN. However, it seems that the vmPFC role is limited to the process of changing the motivational value of the taste since the activity decays when the taste is already familiar and safe. The fact that vmPFC acts modulating accumbens shell activity is supported by the similar activity pattern in both brain areas. Our results are also in accordance with a previous report of increased PrL and IL c-Fos expression associated with CTA extinction [20], thus supporting a general role of the ventral regions of mPFC in flexible behavioural shifting [24]. However, we would like to stress that, as far as we

know, ours is the first study reporting a similar activity pattern specifically in DP. Given that DP maintains bilateral connections with the nucleus of the solitary tract and ipsilateral connections with the parabrachial nucleus as well as interaction with the insular cortex, all of them being areas that integrate taste and visceral inputs contributing to the taste hedonic value, the above proposed role of vmPFC in the modulation of the hedonic shifting of the taste as it becomes familiar can be attributed to DP. Moreover, DP seems to be the most vulnerable mPFC region to the aging impact on AN. While there seems to be a non-significant tendency to reduced PrL and IL activity in the Familiar II older group, a clear different pattern appears in all DP older groups. In comparison with adult groups, older rats exhibit increased DP activity associated with taste novelty. This activity decays as the taste becomes familiar and dramatically decreases when the familiarization process is consolidated. This changing activity pattern does not seem to be attributable to age-related sensory deficits since it changes depending on taste familiarity. Moreover, there is no previous evidence of aging-related taste processing impairments affecting the conventional stimuli used in standard taste learning tasks (Gámiz and Gallo, 2011). This pattern is opposite to that found in the perirhinal cortex of aged rats. Aged rats exhibit lower c-Fos expression than adult rats during the first exposure to vinegar but higher during the sixth exposure [7]. This might suggest a reciprocal inhibitory relationship between both brain areas in taste neophobia and AN so that the decreased perirhinal cortex activity induced by aging would increase DP activity and viceversa. The DP activity pattern of aged rats is also opposite to that exhibited by aged rats in the nucleus accumbens shell, thus supporting reciprocal modulation between both areas. Even though DP shares neuroanatomical characteristics with both PrL and IL [24], research on drug seeking behavior suggests that DP might be functionally distinct [26].

In all, our results support the involvement of vmPFC in the formation of the safe taste memory during the first stages of AN, thus supporting the value of the dorso-ventral division of mPFC. However, a further distinction between ACC, PrL, IL and DP areas unveiled a selective aging impact on DP. This opens new questions on the role of DP in taste memory and prompts research on its interaction with a proposed network mediating neophobia and its attenuation that includes perirhinal cortex, amygdala, insular cortex and accumbens nucleus.

Acknowledgments

Supported by the research project PSI2017-86381-P (MICINN. Spain) and BES-2015-072307 predoctoral fellowship to A. Navarro-Expósito (MECD, Spain). This research is part of the Ph.D. thesis performed by A. N. Expósito at the doctoral program in Psychology of the University of Granada. The authors of this manuscript state that there are no actual or potential conflicts of interest.

References

[1] H.S. Hamezah, L.W. Durani, N.F. Ibrahim, D. Yanagisawa, T. Kato, A. Shiino, S. Tanaka, H.A. Damanhuri, W.Z.W. Ngah, I. Tooyama, Volumetric changes in the aging rat brain and its impact on cognitive and locomotor functions, Exp. Gerontol. 99 (2017) 69–79. https://doi.org/10.1016/j.exger.2017.09.008.

[2] S.H. Fraundorf, K.L. Hourihan, R.A. Peters, A.S. Benjamin, Aging and recognition memory: A meta-analysis, Psychol. Bull. 145 (2019) 339–371. https://doi.org/10.1037/bul0000185.

[3] H. Belblidia, M. Leger, A. Abdelmalek, A. Quiedeville, F. Calocer, M. Boulouard, C. Jozet-Alves, T. Freret, P. Schumann-Bard, Characterizing age-related decline of recognition memory and brain activation profile in mice, Exp. Gerontol. 106 (2018) 222–231. https://doi.org/10.1016/j.exger.2018.03.006.

[4] P. Melo, A. Magalhães, C.J. Alves, M.A. Tavares, L. de Sousa, T. Summavielle, P. Moradas-Ferreira, Methamphetamine mimics the neurochemical profile of aging in rats and impairs recognition memory, Neurotoxicology. 33 (2012) 491–499. https://doi.org/10.1016/j.neuro.2012.03.002.

[5] F. Gámiz, M. Gallo, Taste Learning and Memory: A Window on the Study of Brain Aging, Front. Syst. Neurosci. 5 (2011). https://doi.org/10.3389/fnsys.2011.00091.

[6] I. Morón, M. Gallo, Effect of previous taste experiences on taste neophobia in young-adult and aged rats, Physiol. Behav. 90 (2007) 308–317. https://doi.org/10.1016/j.physbeh.2006.09.036.

[7] B. Gómez-Chacón, E. Morillas, M. Gallo, Altered perirhinal cortex activity patterns during taste neophobia and their habituation in aged rats, Behav. Brain Res. 281 (2015) 245–249. https://doi.org/10.1016/j.bbr.2014.12.020.

[8] M.A. Pelleymounter, M.J. Cullen, Effects of idebenone on information processing in aged Long-Evans rats, Pharmacol. Biochem. Behav. 46 (1993) 415–421. https://doi.org/10.1016/0091-3057(93)90373-2.

[9] A. Grau-Perales, B. Gómez-Chacón, E. Morillas, M. Gallo, Flavor recognition memory related activity of the posterior piriform cortex in adult and aged rats, Behav. Brain Res. 360 (2019) 196–201. https://doi.org/10.1016/j.bbr.2018.12.016.

[10] A.B. Grau-Perales, B. Gómez-Chacón, M. Gallo, Differential activity pattern of c-Fos in the nucleus accumbens between adult and aged rats during flavor recognition memory, Behav. Brain Res. 371 (2019) 111935. https://doi.org/10.1016/j.bbr.2019.111935.

[11] Z. Bimpisidis, M.A.D. Luca, A. Pisanu, G.D. Chiara, Lesion of medial prefrontal dopamine terminals abolishes habituation of accumbens shell dopamine responsiveness to taste stimuli, Eur. J. Neurosci. 37 (2013) 613–622. https://doi.org/10.1111/ejn.12068.

[12] M.C. Gonzalez, M.E. Villar, L.M. Igaz, H. Viola, J.H. Medina, Dorsal medial prefrontal cortex contributes to conditioned taste aversion memory consolidation and retrieval, Neurobiol. Learn. Mem. 126 (2015) 1–6. https://doi.org/10.1016/j.nlm.2015.10.007.

[13] A. Uematsu, A. Kitamura, K. Iwatsuki, H. Uneyama, T. Tsurugizawa, Correlation Between Activation of the Prelimbic Cortex, Basolateral Amygdala, and Agranular Insular Cortex During Taste Memory Formation, Cereb. Cortex. 25 (2015) 2719–2728. https://doi.org/10.1093/cercor/bhu069.

[14] G.A. Mickley, Z. Hoxha, S. Bacik, C.L. Kenmuir, J.A. Wellman, J.M. Biada, A. DiSorbo, Spontaneous recovery of a conditioned taste aversion differentially alters

extinction-induced changes in c-Fos protein expression in rat amygdala and neocortex, Brain Res. 1152 (2007) 139–157. https://doi.org/10.1016/j.brainres.2007.03.050.
[15] K. Shehadi, M. Maroun, Different effects of low frequency stimulation to infralimbic prefrontal cortex on extinction of aversive memories, Brain Res. 1490 (2013) 111–116. https://doi.org/10.1016/j.brainres.2012.10.026.
[16] K. Touzani, R.J. Bodnar, A. Sclafani, Acquisition of glucose-conditioned flavor preference requires the activation of dopamine D1-like receptors within the medial prefrontal cortex in rats, Neurobiol. Learn. Mem. 94 (2010) 214–219. https://doi.org/10.1016/j.nlm.2010.05.009.
[17] D.C. Malkusz, T. Banakos, A. Mohamed, T. Vongwattanakit, G. Malkusz, S. Saeed, S. Martinez, T. Bohn, F. Mahmud, C. Liss, A. Rozvi, K. Touzani, A. Sclafani, R.J. Bodnar, Dopamine signaling in the medial prefrontal cortex and amygdala is required for the acquisition of fructose-conditioned flavor preferences in rats, Behav. Brain Res. 233 (2012) 500–507. https://doi.org/10.1016/j.bbr.2012.05.004.
[18] I. Akirav, V. Khatsrinov, R.-M. Vouimba, M. Merhav, G. Ferreira, K. Rosenblum, M. Maroun, Extinction of conditioned taste aversion depends on functional protein synthesis but not on NMDA receptor activation in the ventromedial prefrontal cortex, Learn. Mem. 13 (2006) 254–258. https://doi.org/10.1101/lm.191706.
[19] M. Maroun, A. Kavushansky, A. Holmes, C. Wellman, H. Motanis, Enhanced Extinction of Aversive Memories by High-Frequency Stimulation of the Rat Infralimbic Cortex, PLOS ONE. 7 (2012) e35853. https://doi.org/10.1371/journal.pone.0035853.
[20] G.A. Mickley, C.L. Kenmuir, A.M. Yocom, J.A. Wellman, J.M. Biada, A role for prefrontal cortex in the extinction of a conditioned taste aversion, Brain Res. 1051 (2005) 176–182. https://doi.org/10.1016/j.brainres.2005.05.033.
[21] J.M. Juraska, N.C. Lowry, Neuroanatomical Changes Associated with Cognitive Aging, in: M.-C. Pardon, M.W. Bondi (Eds.), Behav. Neurobiol. Aging, Springer, Berlin, Heidelberg, 2012: pp. 137–162. https://doi.org/10.1007/7854_2011_137.
[22] D. Öngür, J.L. Price, The Organization of Networks within the Orbital and Medial Prefrontal Cortex of Rats, Monkeys and Humans, Cereb. Cortex. 10 (2000) 206–219. https://doi.org/10.1093/cercor/10.3.206.
[23] G. Paxinos, C. Watson, The rat brain in stereotaxic coordinates, Sydney : Academic Press, 1982. https://trove.nla.gov.au/work/8025602 (accessed January 16, 2020).
[24] C.A. Heidbreder, H.J. Groenewegen, The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics, Neurosci. Biobehav. Rev. 27 (2003) 555–579. https://doi.org/10.1016/j.neubiorev.2003.09.003.
[25] C. Inui-Yamamoto, T. Yamamoto, K. Ueda, M. Nakatsuka, S. Kumabe, T. Inui, Y. Iwai, Taste preference changes throughout different life stages in male rats, PloS One. 12 (2017) e0181650. https://doi.org/10.1371/journal.pone.0181650.
[26] A.L. Willcocks, G.P. McNally, The role of medial prefrontal cortex in extinction and reinstatement of alcohol-seeking in rats, Eur. J. Neurosci. 37 (2013) 259–268. https://doi.org/10.1111/ejn.12031.

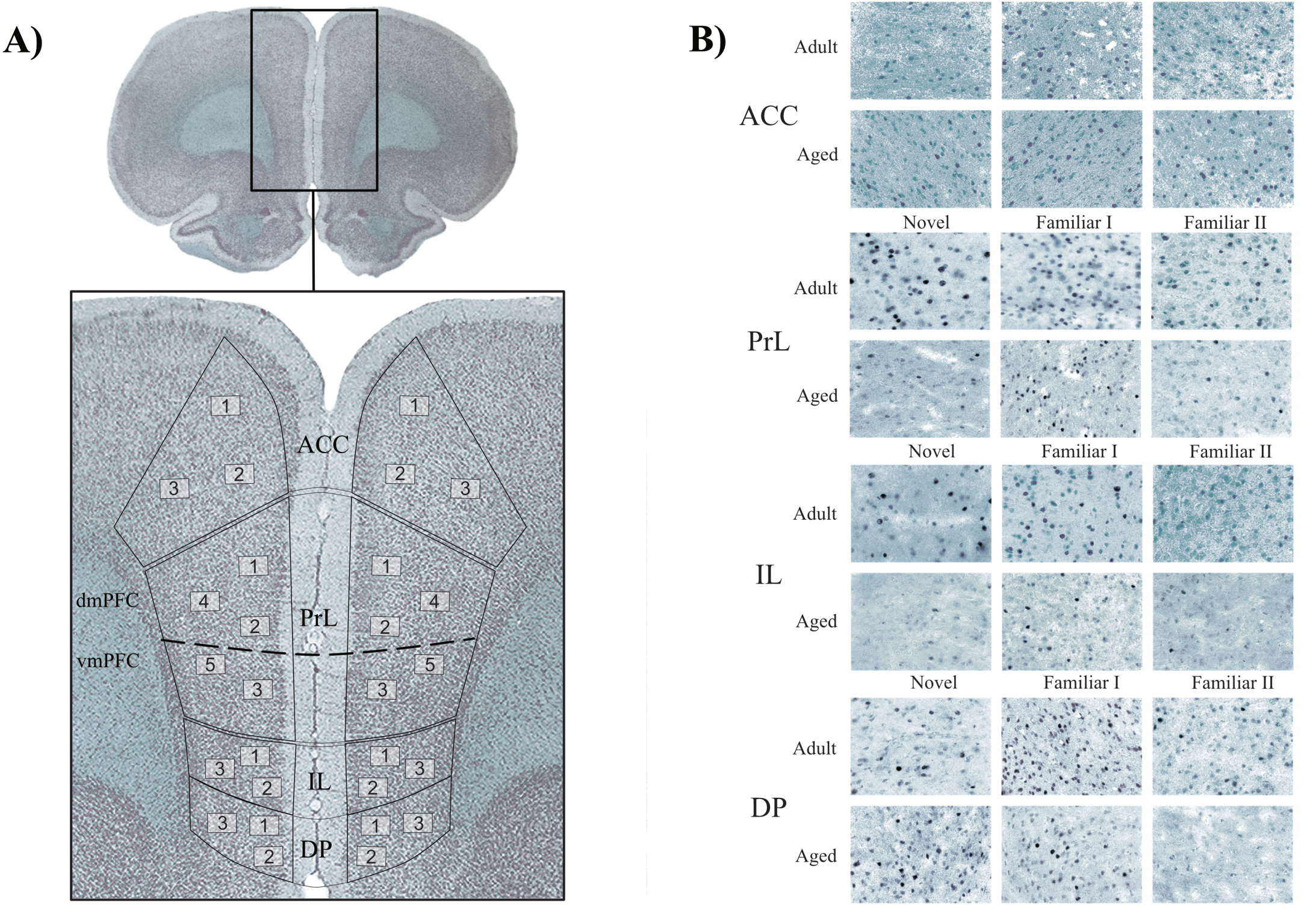
Figure legends

Figure 1. A) Location of the microphotographs (100 microns, 40X) covering the regions of the mPFC assessed. The images were numbered following a dorso-ventral and medio-lateral axis. B) Representative microphotographs showing c-Fos positive cells in the anterior cingulate cortex (ACC), prelimbic cortex (PrL), infralimbic cortex (IL) and dorsal peduncular cortex (DP) of adult and aged rats belonging to the three familiarity groups (Novel, Familiar I and Familiar II).

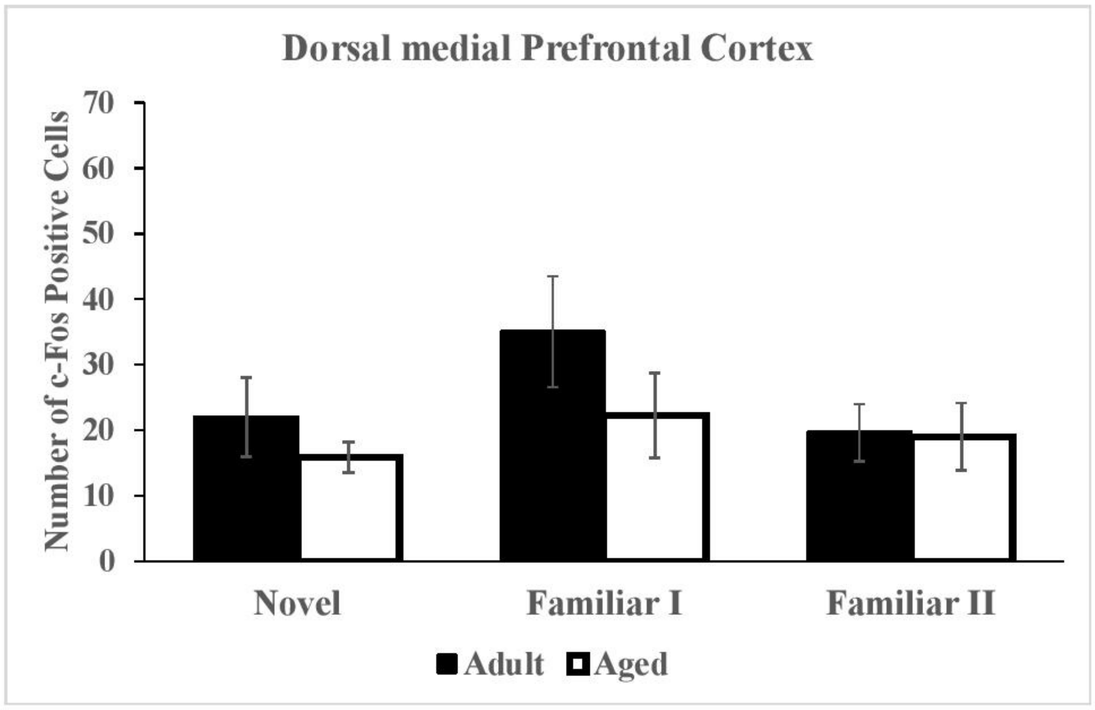
Figure 2. Mean (±SEM) number of c-Fos positive cells in the mPFC regions according to a) dorsal versus ventral subdivision b) anterior cingulate cortex (ACC), prelimbic cortex (PrL), infralimbic cortex (IL) and dorsal peduncular cortex (DP). * vs. Familiar I group; # adult vs. aged groups.

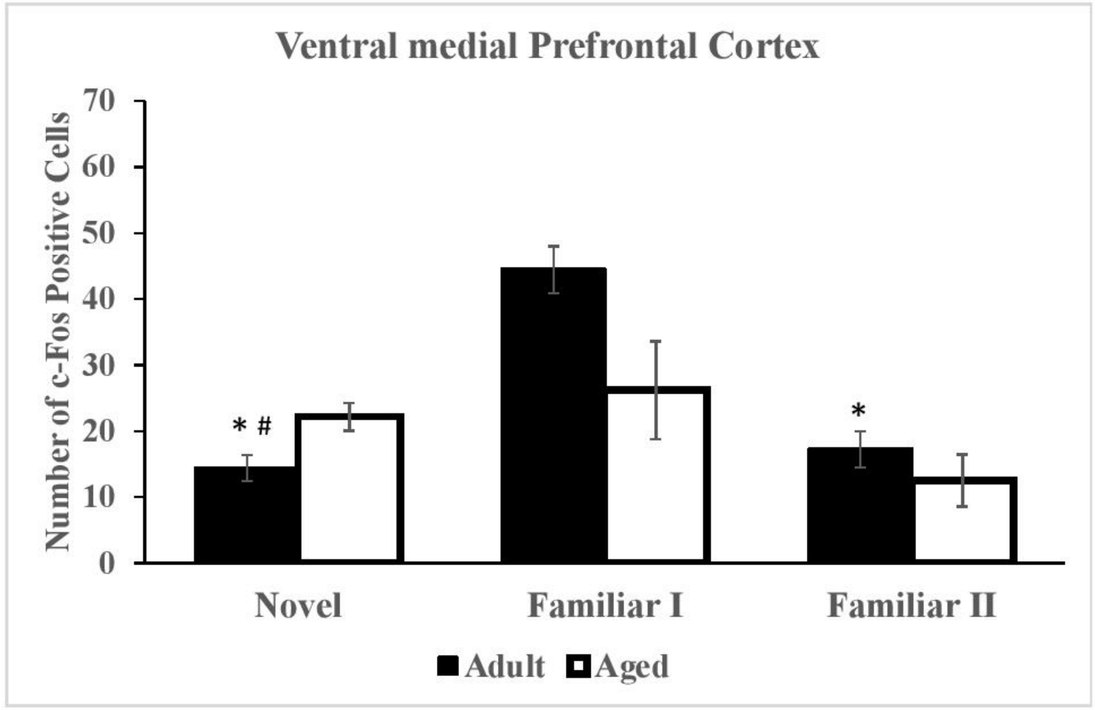
Table 1.
Mean (± SEM) vinegar consumption (ml.) of the different adult and aged groups.

	Novel	Familiar I	Familiar II
Adult	6.32 (± 0.53)	9.64 (± 0.87)	11.73 (± 0.78)
Aged	5.65 (± 0.8)	7.01 (± 0.8)	11.51 (± 0.79)

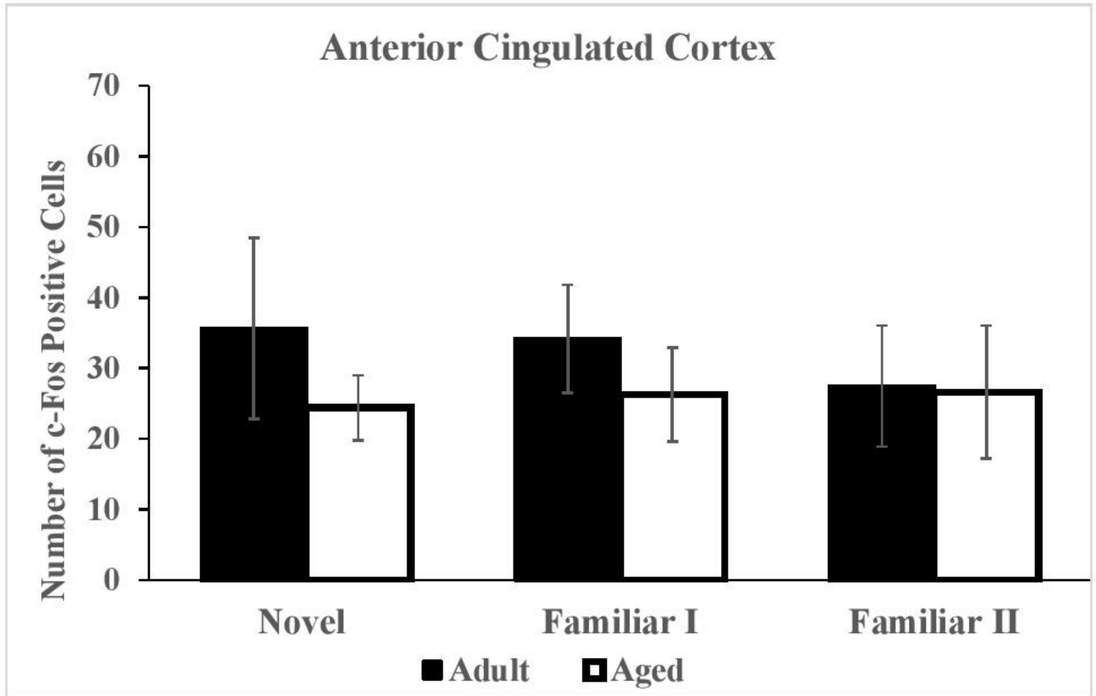

A)
ACC
PrL
IL
DP
dmPFC
vmPFC
B)
Novel
Familiar I
Familiar II
Adult
Aged
ACC
PrL
IL
DP

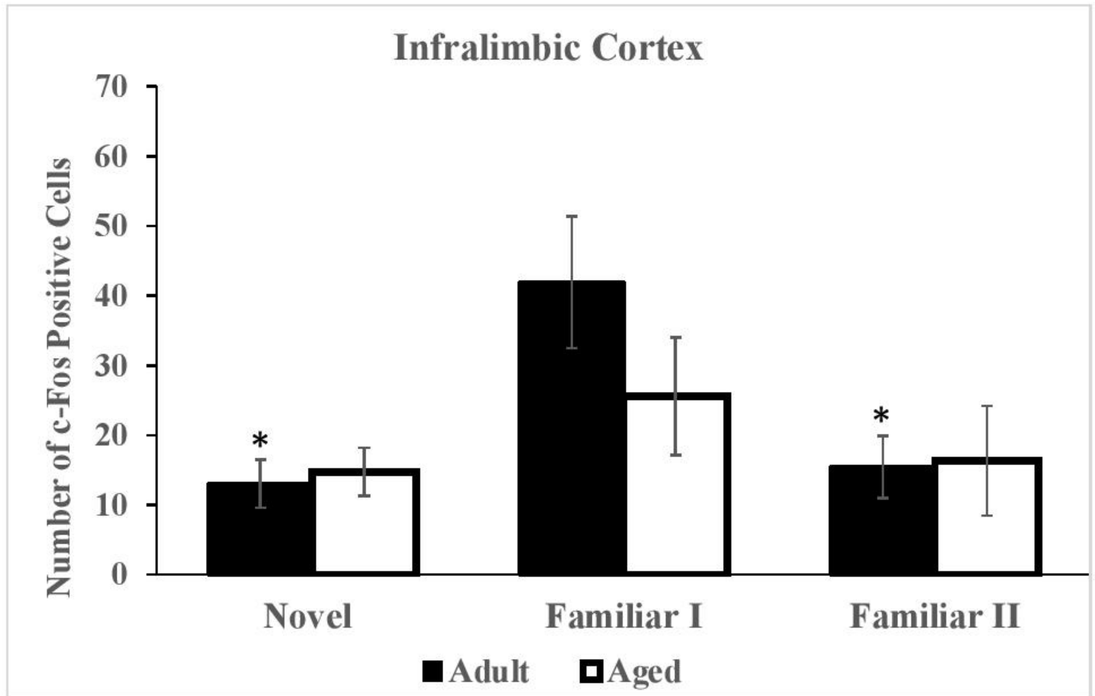
A)

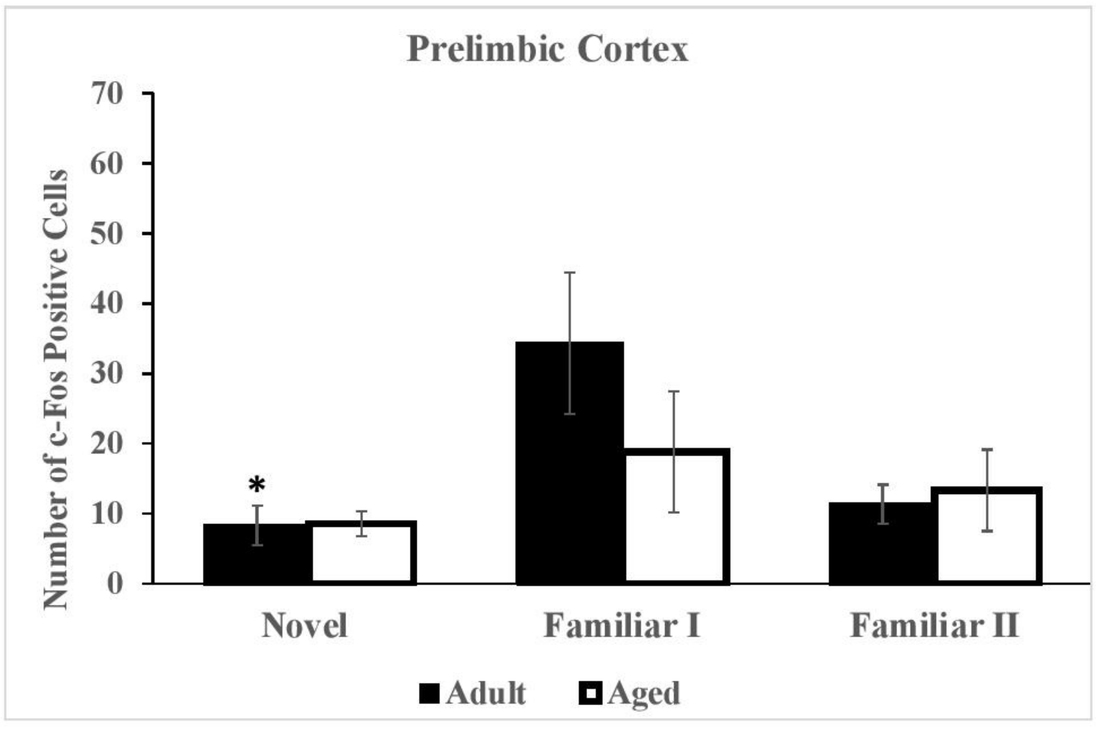

Dorsal medial Prefrontal Cortex
Number of c-Fos Positive Cells
70
60
50
40
30
20
10
0
Novel
Familiar I
Familiar II
Adult
Aged

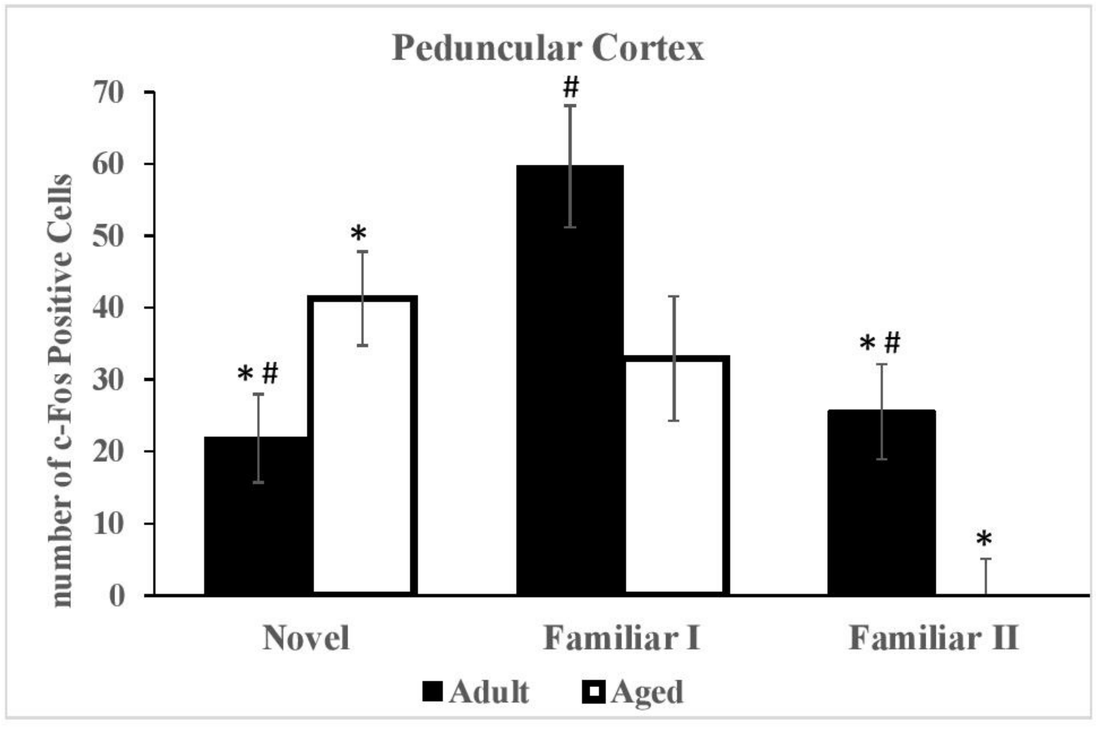

Ventral medial Prefrontal Cortex
Number of c-Fos Positive Cells
70
60
50
40
30
20
10
0
* #
*
Novel
Familiar I
Familiar II
Adult
Aged

B)


Anterior Cingulated Cortex
Number of c-Fos Positive Cells
70
60
50
40
30
20
10
0
Novel
Familiar I
Familiar II
Adult
Aged


Infralimbic Cortex
Number of c-Fos Positive Cells
70
60
50
40
30
20
10
0
*
*
Novel
Familiar I
Familiar II
Adult
Aged


Prelimbic Cortex
Number of c-Fos Positive Cells
70
60
50
40
30
20
10
0
*
Novel
Familiar I
Familiar II
Adult
Aged


Peduncular Cortex
number of c-Fos Positive Cells
70
60
50
40
30
20
10
0
#
*
* #
* #
*
Novel
Familiar I
Familiar II
Adult
Aged