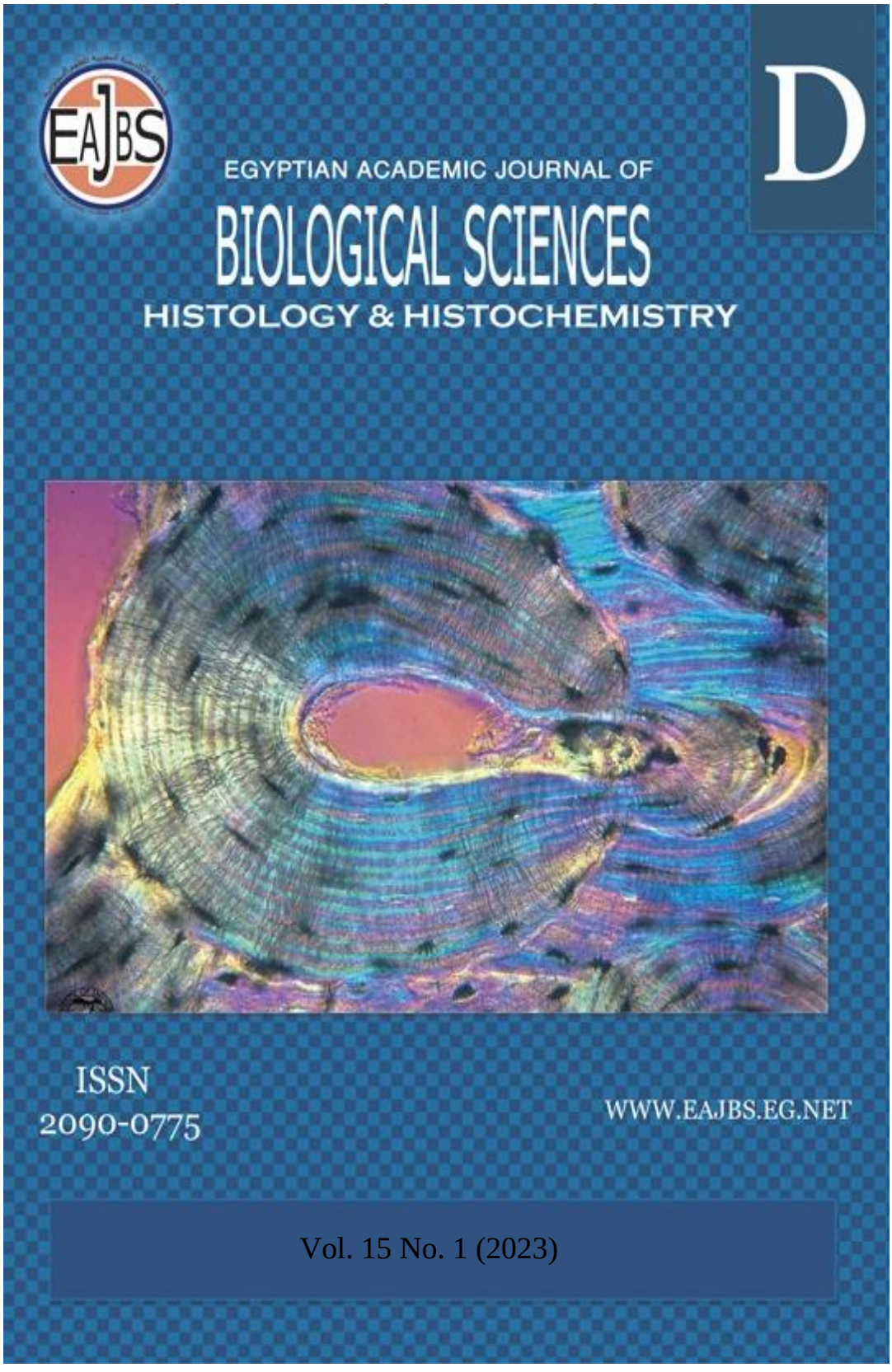

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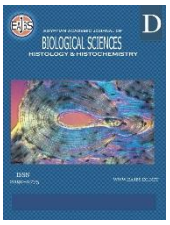

Circadian and Age-Related Changes in the Expression of NeuN and p42/44-MAPK in Mouse Amygdaloid Complex

Amira A. H. Ali[1,2]; and Ahmed Nabawy Ahmed Nasr[1]

1-Department of Human Anatomy and Embryology, Faculty of Medicine, Mansoura University, Egypt.

2- Institute of Anatomy II, Medical Faculty, Düsseldorf University, Germany.

E.Mail : amira.aminhassan.ali@gmail.com,- ahmednabawy@mans.edu.eg

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ABSTRACT

The amygdala is an essential brain region responsible for social behaviors and anxiety. The lateral amygdaloid nucleus (LAN), basal amygdaloid nucleus (BAN) and medial amygdaloid nucleus (MAN) are important nuclei in the amygdaloid complex. There is a change of emotional responses such as anxiety over the course of the day, thus called circadian (circa: about, diem: day) as well as during the process of aging indicating a regulation of the amygdalar function via the biological clock, which is also affected by aging. Thus, we investigated the circadian changes between the early light phase and early dark phase of the day in addition to age-related changes between young and middle-aged mice on the expression of the neuronal nuclear protein (NeuN) and the neural plasticity marker phosphorylated mitogen-activated protein kinase 1/2 (p42/44-MAPK) in the LAN, BAN and MAN subregions of the amygdala using immunohistochemistry.

We show higher expression levels of NeuN and p42/44-MAPK during the light phase indicating a strong correlation between the neuronal activity in the amygdala and the circadian system. From young to middle-aged mice, the expression levels of these neuronal proteins in the amygdala decrease, suggesting a pronounced interaction between the circadian system and aging in the regulation of the neuronal functional state in the amygdala. Our data may present a better understanding of the role of circadian timing and the effect of age on the neuronal functions within the amygdala and related behavioral changes such as fear and anxiety in health and disease.

INTRODUCTION

The amygdala is a crucial part of the circuitry responsible for fear-related behaviors and anxiety (Fernando *et al.,* 2013, Gothard, 2020). Disorders in the amygdala lead to altered processing of fear-memory trace, which may underlie some anxiety disorders for instance posttraumatic stress (Sah *et al.*, 2003). The amygdala is positioned in the medial temporal lobe (Ressler, 2010) and amygdaloid complex consists of diverse 13 nuclei including the lateral amygdaloid nucleus (LAN), basal amygdaloid nucleus (BAN) and medial amygdaloid nucleus (MAN) (Gothard, 2020, AbuHasan et al., 2022). LAN receives sensory and nociceptive fear-related information while BAN receives contextual fear information from the hippocampal formation. Both LAN and BAN are tightly interconnected and referred to as basolateral complexes. Output fibers project to the prefrontal cortex and hippocampus to mediate fear-related memory circuits among amygdala, prefrontal cortex and hippocampus (Albrecht and Stork, 2017, Lisk *et al.*, 2020).

The man is an essential structure in social and sexual behaviors.It receives input from the olfactory bulb and sends output to other amygdaloid nuclei, hypothalamus and bed nucleus of stria terminalis (Pardo-Bellver *et al.*, 2012, Moreno-Santos *et al.*, 2021). In humans, the size of the amygdala is related positively to social enrichment, emphasizing its essential role in social behavior (Bickart *et al.*, 2011), and it is also involved in sexual orientation (Swaab, 2007, AbuHasan et al., 2022). Dysfunction of the amygdala is correlated with mental disorders including obsessive-compulsive disorder and anxiety disorders (Arehart-Treichel, 2014).

There is a change in emotional responses regulated by the amygdala such as anxiety and passive avoidance over the course of the day (Meseguer Henarejos *et al.*, 2020). This implies modulation of the amygdalar function by the biological circadian clock (Albrecht and Stork, 2017), which regulates the physiology and behavior according to the twenty-four-hour solar day, thus called circadian (circa: about, diem: day) (Ruan *et al.*, 2021). The circadian rhythms are orchestrated by the central pacemaker in the suprachiasmatic nucleus (SCN) of the hypothalamus. SCN receives photic input from the retina in addition to non-photic cues e.g. activity and arousal levels and sends this information to other brain areas and body organs to synchronize the rhythms in physiology and behavior in a 24-h rhythmic pattern (Reppert and Weaver, 2002). Within the amygdala, this is likely regulated intrinsically via the oscillation of circadian clock genes (Savalli *et al.*, 2014) and proteins rhythms (Ramanathan et al., 2010) or extrinsically through circadian variation of the glucocorticoid levels that influence the neuronal activity of amygdala (den Boon *et al.*, 2019).

Aging induces detrimental effect on various brain structures and, subsequently, impacts their functions including cognition decline and emotional changes e.g increased anxiety (Przybysz *et al.*, 2020). Previous studies showed that aging results in morphological changes in the amygdala including decreased dendritic total length and complexity accompanied by increased anxiety-like behavior (Sotoudeh *et al.*, 2022). In addition, amygdalar neural activity decreases with age advance in males (Haider *et al.*, 2021).

However, the age-related changes from young to middle age in the amygdala and the age-related effects on circadian neuronal plasticity is not yet clear. The identification of the impact of age on neuronal plasticity may provide a better understanding of age-related neuropathological and functional changes and thus, may help develop new therapeutic strategies for age-dependent neurodegenerative diseases.

The neuronal nuclear protein (NeuN) is a neuronal-specific protein that is expressed exclusively in the nucleus and in the perinuclear cytoplasm of most mature neurons (Petrova *et al.*, 2014, Verdiev *et al.*, 2009). NeuN is highly expressed in the nucleus of neurons in brain regions containing less chromatin density (Lind *et al.*, 2005), constrained to the nuclear matrix (Dent *et al.*, 2010) and plays a modulatory role in the regulation of the neuronal functions (Mullen *et al.*, 1992).

Additionally, during fear acquisition, neuronal activation induces the elevation of intracellular calcium with subsequent activation of cyclic adenosine monophosphate (cAMP) (Schafe *et al.*, 2001). This is followed by the triggering of protein kinases including mitogen-activated protein kinase (MAPK), which induces phosphorylation of the cAMP response element-binding protein (CREB) and thus, influences the transcription of synaptic plasticity genes related to fear to condition (Cestari et al., 2014). Thus, activated MAPK represents a promising target for psychiatric disorders treatment

The present study aims to investigate if the expression of the neuronal nuclear protein (NeuN) and the neural plasticity marker p42/44-MAPK is different during the early day-time than the early night-time in the subregions of the amygdala. The present study also attempts to reveal the age-dependent alteration of the expression of these markers in the subregion of the amygdala including the lateral amygdaloid nucleus (LAN), medial amygdaloid nucleus (MAN) and basal amygdaloid nucleus (BAN). Such findings will provide a better understanding of the role of circadian timing and the effect of age on the neuronal functions within the amygdala and related behavioral changes such as fear and anxiety.

MATERIALS AND METHODS

1. Animals:

24 Male C57Bl/6 mice were used including "young" mice that were one month old (n=12 mice), and "middle-aged" mice that were 10 months old (n=12 mice), according to a previous report (Dutta and Sengupta, 2016). Mice were kept in groups of 2-3 in standard cages on a normal 12h/12h light/dark schedule. The light was turned on at 06.00 am [= Zeitgeber time (ZT)00] and turned off at 06.00 pm [= Zeitgeber time (ZT)12]. Mice were housed under controlled temperature and humidity conditions with free access to chow and water ad libitum for two weeks for acclimatization. All experiments were approved by the Mansoura University Animal Care and Use Committee [approval number: MU-ACUC (MED.R.22.11.7)] and were conducted in accordance with the guidelines for the care and use of laboratory animals of the National Institutes of Health.

2. Tissue Processing:

One group of young (n=6 mice) and middle-aged mice (n=6 mice) were sacrificed during the daytime (two hours after light on =ZT02), while another group of young (n=6 mice) and middle-aged mice (n=6 mice) was sacrificed during the nighttime (two hours after light off =ZT14). Mice were deeply anesthetized via single pentobarbital intraperitoneal injection at a dose of (40mg/kg). Intracardiac perfusion with phosphate-buffered saline followed by 4% paraformaldehyde was done. The brains were dissected from the skull and were post-fixed by immersion in 4% paraformaldehyde for additional 24 hours and processed by routine histopathological examinations using the paraffin method.

3. Immunohistochemistry:

The formaldehyde-fixed brain samples were dehydrated in graded alcohol, cleared with xylol, and then fixed in wax. Paraffin sections of 5 µm thick coronal sections were obtained by a rotatory microtome. The sections between Bregma -1.46 to -2.18 according to Paxinos Mouse Brain Atlas and were selected for the staining (Paxinos and Keith B. J. Franklin, 2007).

The coronal sections were deparaffinized and rehydrated as described above. Then, to block endogenous peroxidase, the sections were incubated with 10%-H_2O_2 for 15 min. The sections were rinsed with phosphate-buffered saline (PBS) at room temperature three times each 5 min. The sections were then incubated for 1 hour with 1%BSA and 5% goat normal serum in PBS-triton. This was followed by incubation with primary antibodies; rabbit anti-NeuN (1:1000, Abcam) and rabbit anti p42/44-MAPK (1:1000, Cell Signaling Technology) at 4°C overnight. Next, sections were rinsed using PBS-triton three times each for 5 minutes. Sections were then incubated with anti-rabbit biotin-conjugated secondary antibody (1:500, Abcam) for one hour at room temperature. The sections were then rinsed with PBS-Triton and then incubated with the Avidin-Biotin-Complex kit (1:200, DETHP1000, Sigma-Aldrich) for another hour at room temperature. This was followed by rinsing with PBS-triton and then by incubation in 0.05% 3,3'-diaminobenzidine for 5 minutes at RT. Sections were finally washed with PBS and coverslipped.

4. Image Acquisition:

The images of stained sections were acquired using the Olympus® CX41 light microscope connected to the Olympus® SC100 digital camera using bright field mode. The experimental conditions were coded during image acquisition and obscured during analysis to avoid bias. The conditions for image acquisition and analysis were kept consistent throughout the whole experiment set.

The amygdala was investigated in three regions based on Paxinos mouse atlas: 1. Lateral amygdaloid nucleus (LAN), 2. Basal amygdaloid nucleus (BAN), 3. Medial amygdaloid nucleus (MAN). Equivalent fields in amygdala from all groups were analysed. The regions of interest were delineated and the immunoreactivity above the background intensity of NeuN was quantitively estimated and shown as arbitrary units (a.u.) using ImageJ software (http://rsbweb,nih.gov/ij). The p-ERK immunoreactive cells were counted in a delineated area in the individual subregions of the amygdala) using ImageJ software (http://rsbweb,nih.gov/ij), then the mean cell density was estimated and shown as the number of cells/mm^2.

5.Statistical Analysis:

GraphPad Prism 8.3.0 software was used for statistical analysis. T-tests were used to analyze significant differences between the two groups. Data are expressed as mean ± standard error of the mean (SEM). The results were regarded as statistically significant if $P < 0.05$.

RESULTS

1.Circadian Changes in NeuN Expression in Amygdala of Young Mice:

In one-month-old mice, the expression of neuronal marker NeuN in the amygdala was significantly increased during the day-time (ZT02) (Fig. 1A, B) as compared to the nighttime (ZT14) in the BAN ($p = 0.02$) and in the MAN ($P = 0.03$) (Fig. 1C, D). However, the expression of NeuN in the LAN was not significantly changed between day-time (ZT02) and night-time (ZT14) ($p > 0.05$) (Fig. 1E).

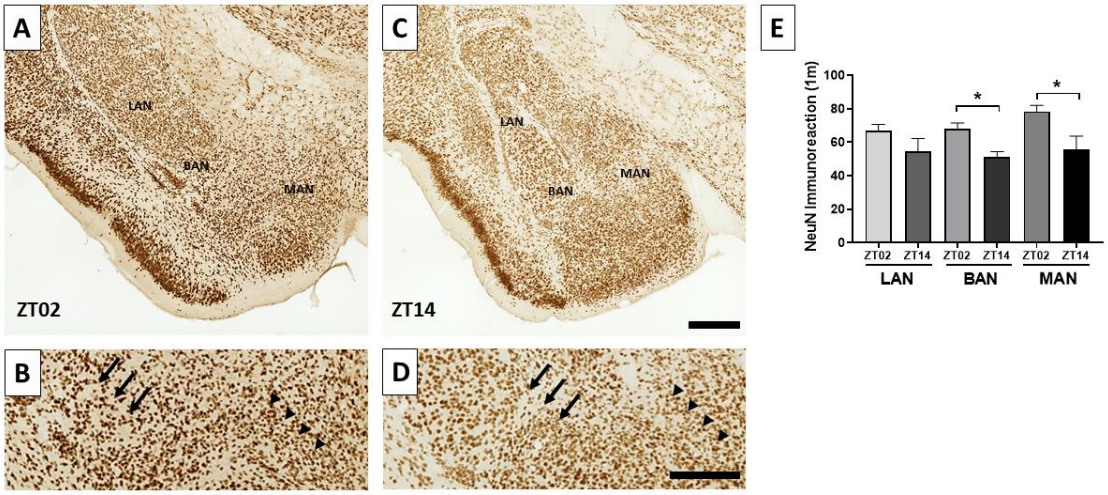

Fig. 1. Representative photomicrographs of NeuN immunohistochemically-stained coronal sections of brain tissue obtained from 1-month-old mice (1m). (A) Low magnification of the amygdala complex including the lateral amygdaloid nucleus (LAN), basal amygdaloid nucleus (BAN) and medial amygdaloid nucleus (MAN) stained against neuronal marker NeuN during the early day-time [(Zeitgeber time) ZT02 = two hours after the light on]. (B) High magnification photomicrographs showing NeuN-positive immunoreaction (brown staining) of the cell nuclei in BAN (black arrows) and MAN (black arrowheads) during the early daytime. (C) Low magnification of the amygdala complex stained against neuronal marker NeuN during the early night-time (ZT14 = 2 hours after the light off). (D) High magnification photomicrographs showing NeuN-positive immunoreaction (brown staining) of the cell nuclei in BAN (black arrows) and MAN (black arrowheads) during the early night-time. (E) Quantification of NeuN immunoreaction in LAN, BAN and MAN during the early day-time and early night-time shows significant differences between the day-time and night-time in BAN and MAN. *: $p < 0.05$ using Student-t-test. Scale bar = 100µm in A, C. Scale bar = 50µm in B, D.

2.Circadian Change in NeuN Expression in Amygdala of Middle-Aged Mice:

In ten-month-old mice, the expression of the neuronal marker NeuN in the amygdala was significantly increased during the day-time (ZT02) (Fig. 2A, B) as compared to the nighttime (ZT14) only in the BAN ($p = 0.02$) (Fig. 2C, D). However, the expression of NeuN in the LAN and in the MAN was not significantly changed between day-time (ZT02) and night-time (ZT14) ($p > 0.05$) (Fig. 2E). We found also age-dependent changes in NeuN expression amygdala as the expression of NeuN was higher in the young mice than in middle-aged mice, in particular, during the day-time in LAN ($p = 0.02$), BAN ($p = 0.02$) and in the MAN ($p = 0.003$). Nevertheless, there were no significant age-dependent changes in the expression of NeuN during the night-time (ZT14) in the LAN, in BAN, or in MAN ($p > 0.05$) (Fig. 2F).

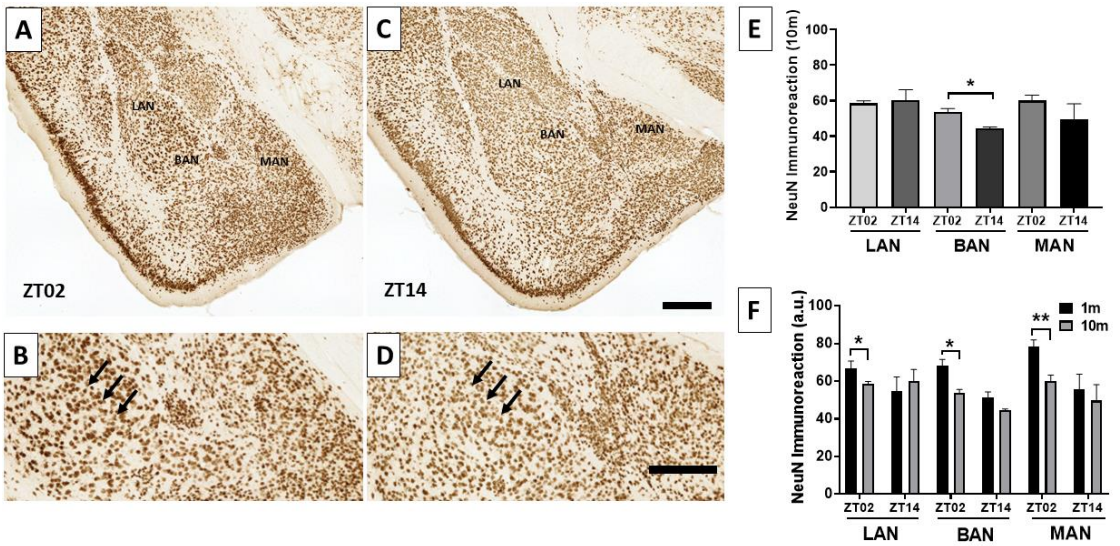

Fig. 2. Representative photomicrographs of immunohistochemically-stained coronal sections of brain tissue obtained from 10-month-old mice (10m). (A) Low magnification of the amygdala complex including the lateral amygdaloid nucleus (LAN), basal amygdaloid nucleus (BAN) and medial amygdaloid nucleus (MAN) stained against neuronal marker NeuN during the early day-time [(Zeitgeber time) ZT02 = two hours after the light on]. (B) High magnification photomicrographs showing NeuN-positive immunoreaction (brown staining) of the cell nuclei in BAN (black arrows) during the early daytime. (C) Low magnification of the amygdala complex stained against neuronal marker NeuN during the early night-time (ZT14 = 2 hours after the light off). (D) High magnification photomicrographs showing NeuN-positive immunoreaction (brown staining) of the cell nuclei in BAN (black arrows) during the early night-time. (E) Quantification of NeuN immunoreaction in LAN, BAN and MAN during the early day-time and early night-time shows significant differences between the day-time and night-time in BAN. (F) Quantification of NeuN immunoreaction (a.u.: arbitrary units) in LAN, BAN and MAN during the early day-time and early night-time in 1m mice (black bars) and 10 m mice (gray bars) showing significant differences between 1m and 10m mice during the day-time in LAN, BAN and MAN. *: $p < 0.05$, **: $p < 0.01$ using Student-t-test. Scale bar = 100µm in A, C. Scale bar = 50µm in B, D.

3.Circadian Change in p42/44-MAPK Expression in Amygdala of Young Mice:

The p42/44-MAPK immunoreactive cells were found in the amygdala as indicated by brown staining, which was mainly obvious in the cell nuclei. In one-month-old mice, the expression of p42/44-MAPK in the amygdala was significantly increased during the day-time (ZT02) (Fig.3A-C) as compared to the nighttime (ZT14) (Fig. 3D-F) in the LAN ($P = 0.03$), BAN ($p = 0.04$) and in the MAN ($P = 0.02$) (Fig. 3G).

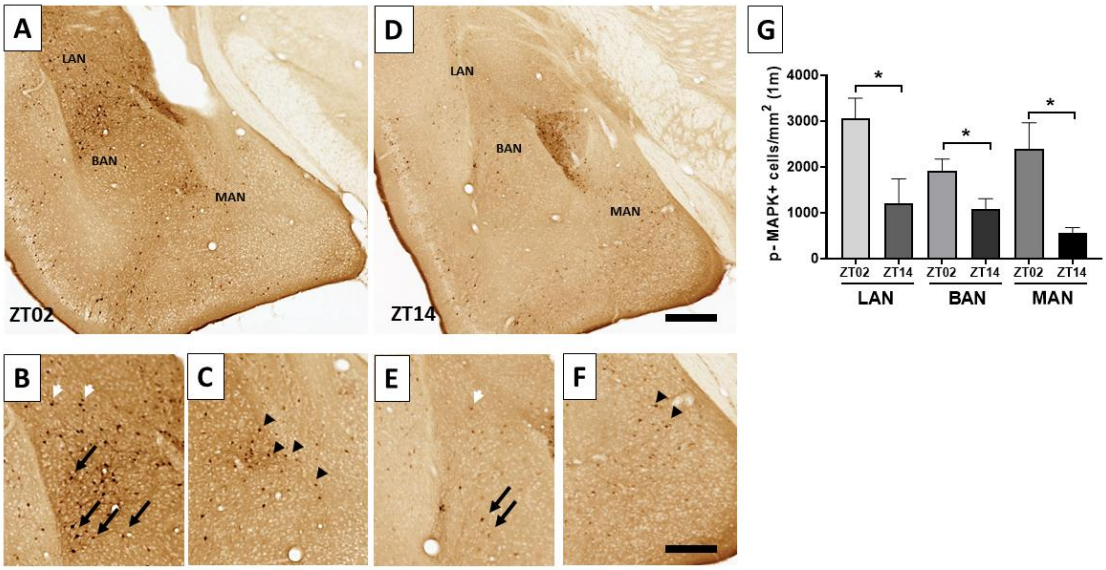

Fig. 3. Representative photomicrographs of immunohistochemically-stained coronal sections of brain tissue obtained from 1-month-old mice (1m). (A) Low magnification of the amygdala complex including the lateral amygdaloid nucleus (LAN), basal amygdaloid nucleus (BAN) and medial amygdaloid nucleus (MAN) stained against neuronal plasticity marker p42/44-MAPK (p-MAPK) during the early day-time [(Zeitgeber time) ZT02 = two hours after the light on]. High magnification photomicrographs showing p-MAPK positive immunoreaction (brown staining) of the cell nuclei in (B) LAN (white arrowheads), BAN (black arrows) and (C) MAN (black arrowheads) during the early day-time. (D) Low magnification of the amygdala complex stained against neuronal plasticity marker p42 44-MAPK (p-MAPK) during the early night-time (ZT14 = 2 hours after the light off). High magnification photomicrographs showing neuronal p-MAPK positive immunoreaction (brown staining) of the cell nuclei in (E) LAN (white arrowheads), BAN (black arrows) and (F) MAN (black arrowheads) during the early night-time. (G) Quantification of p-MAPK positive cells/mm^2 in LAN, BAN and MAN during the early day-time and early night-time shows significant differences between the day-time and night-time in LAN, BAN and MAN. *: $p < 0.05$ using Student-t-test. Scale bar = 100µm in A, D. Scale bar = 50µm in B, C, E, F.

4.Circadian Change in p42/44-MAPK Expression in Amygdala of Middle-Aged Mice:

In ten-month-old mice, the p42/44-MAPK immunoreactive cells were also found in the amygdala as indicated by brown staining, which was remarkably localized in the cell nuclei.

The expression of p42/44-MAPK in the amygdala was significantly increased during the day-time (ZT02) (Fig. 4A, B) as compared to the nighttime (ZT14) (Figure 4D, E) in the LAN ($p = 0.03$) and in the BAN ($p = 0.03$). However, the expression of p42/44-MAPK in the MAN was not significantly changed between day-time (ZT02) (Fig, 4C) and night-time (ZT14) (Figure 4F) ($p > 0.05$) (Figure 4G). We found also age-dependent changes in p42/44-MAPK expression in amygdala as the expression of p42/44-MAPK was higher in the young mice while the expression decreased in the middle-aged mice, in particular, during the day-time in the MAN ($p = 0.02$). Nevertheless, there were no significant age-dependent changes neither in the expression of p42/44-MAPK in the LAN nor in BAN ($p > 0.05$) during the daytime or during the nighttime. In addition, there was no difference between the young and the middle-aged mice during the night-time (ZT14) MAN ($p > 0.05$) (Fig. 4 H).

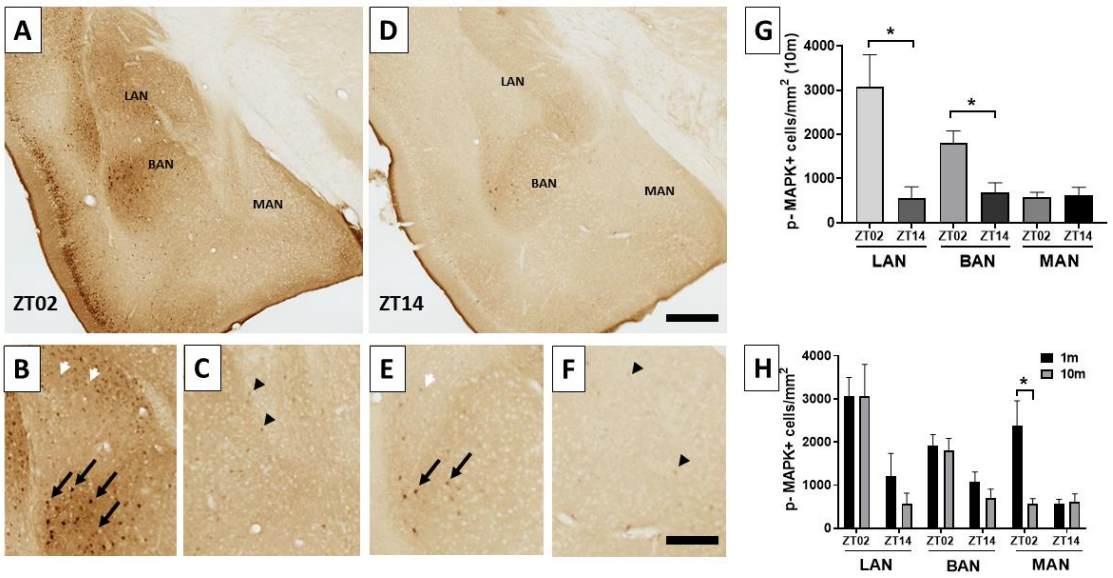

Fig. 4. Representative photomicrographs of immunohistochemically-stained coronal sections of brain tissue obtained from 10-month-old mice (10m). (A) Low magnification of the amygdala complex including the lateral amygdaloid nucleus (LAN), basal amygdaloid nucleus (BAN) and medial amygdaloid nucleus (MAN) stained against neuronal plasticity marker p42/44-MAPK (p-MAPK) during the early day-time [(Zeitgeber time) ZT02 = two hours after the light on]. High magnification photomicrographs showing p-MAPK positive immunoreaction (brown staining) of the cell nuclei in (B) LAN (white arrowheads), BAN (black arrows) and (C) MAN (black arrowheads) during the early day-time. (D) Low magnification of the amygdala complex stained against neuronal plasticity marker p-MAPK during the early night-time (ZT14 = 2 hours after the light off). High magnification photomicrographs showing neuronal p-MAPK positive immunoreaction (brown staining) of the cell nuclei in (E) LAN (white arrowheads), BAN (black arrows) and (F) MAN (black arrowheads) during the early night-time. (G) Quantification of p-MAPK positive cells/mm2 in LAN, BAN and MAN during the early day-time and early night-time shows significant differences between the day-time and night-time in LAN and BAN. (H) Quantification of p-MAPK positive cells/mm2 in LAN, BAN and MAN during the early day-time and early night-time in 1m mice (black bars) and 10 m mice (gray bars) showing significant differences between 1m and 10m mice during the day-time in MAN. *: $p < 0.05$ using Student-t-test. Scale bar = 100µm in A, D. Scale bar = 50µm in B, C, E, F.

DISCUSSION

In this study, we report a circadian change in the expression of neuronal marker NeuN and the plasticity marker p42/44-MAPK in the amygdala in a subregion-specific manner and being more robust in young mice than in the middle-aged. In addition, the expression of NeuN and p42/44-MAPK is subjected to a significant reduction in middle-aged mice as compared to young mice, indicating an age-dependent effect.

Alteration of NeuN expression, distribution and protein concentration is associated with changes in neuronal functional state and integrity, as it has been shown that NeuN expression changes during neuronal stimulation (Weyer and Schilling, 2003). In addition, pathological conditions and neuronal injuries may impact NeuN expression levels (McPhail *et al.,* 2004). For instance, the expression of NeuN is decreased in the striatum following ischemia (Korzhevskii *et al.,* 2009) and in Huntington's disease (Tippett *et al.,* 2007) as well as in hypoxia and after brain injury (Unal-Cevik *et al.,* 2004). Changes in NeuN expression levels may rely on the alteration of post-translational modifications (Gusel'nikova and Korzhevskiy, 2015).

The entire range of functions of the NeuN protein in cells has not been determined (Gusel'nikova and Korzhevskiy, 2015). However, it has been shown that NeuN is implied in neuronal activity (Hight *et al.,* 2010, Mullen *et al.,* 1992). NeuN seems to be regulated by the circadian system in a region-specific manner. For instance, there is a higher expression of NeuN in the somatosensory cortex during the dark phase while the expression of NeuN in the visual cortex during the light phase dramatically increases (Hight *et al.,* 2010). These reports are in line with our demonstration of significant differences in NeuN expression levels in the amygdala between day-time and night-time. This is also consistent with circadian regulation of other neuronal activity markers for example c-FOS transcription factor protein in various brain regions either through a direct effect of light (Öztürk *et al.,* 2021) or due to behavioral activity e.g. locomotor activity and memory tasks (Cho *et al.,* 2017). NeuN expression is reported to be affected by aging, consistent with our results as we showed that the expression of NeuN in the amygdala decreases with age and significantly differs between young and middle-aged mice. A recent study has demonstrated that NeuN dramatically decreases in the anterior frontal cortex and in the hippocampus during normal and premature aging, while other brain areas including hypothalamus and motor cortex were less influenced (Garrido *et al.,* 2021). In contrast, another study on the hippocampus showed no significant differences among young, adult and aged groups neither in mice nor in rats (Ahn *et al.,* 2016). A limitation of Ahn et al. is that they considered only the NeuN+ cell count but not the immunoreaction/ protein expression levels.

Next, we analysed the expression of phosphorylated mitogen-activated protein kinase 1/2 (p44/42-MAPK), also known as an extracellular signal-regulated kinase (ERK1/2), which is a crucial intracellular pathway that modulates a wide variety of neuronal functions (Roskoski, 2012) such as learning and memory, anxiety, neuronal plasticity and neuronal regeneration (Haghparast *et al.,* 2014)

Activation of p42/44-MAPK pathway leads to the triggering of downstream targets including phosphorylation of cyclic AMP responsive element-binding protein (CREB) (Qi *et al.,* 2008) and activation of c-FOS transcription factor (Cruz *et al.,* 2007) that regulates the neuronal activity (Azieva *et al.,* 2021, Nathaniel *et al.,* 2012). p44/42-MAPK is expressed in the amygdala and its expression levels significantly increase following fear conditioning presumably due to N-methyl-D-aspartate receptor (NMDA) receptors signaling (Bertotto *et al.,* 2011). Recent studies showed that suppression of p44/42-MAPK pathway induces fear memory deficits (Zhao *et al.,* 2021, de Carvalho *et al.,* 2021) and impaired defensive responses. These data point to the functional role of MAPK in the amygdala. We demonstrated an increase in the p42/44-MAPK expression levels in the amygdala in young and middle-aged mice, which was more remarkable in young mice. This circadian expression was lost in middle-aged mice, indicating an interaction of aging, circadian clock and p42/44-MAPK pathway. In agreement with our findings, p44/42-MAPK shows differential expression throughout the day with peak expression during the light phase while trough expression levels were noticed during the dark phase within the suprachiasmatic nucleus (Öztürk *et al.,* 2021). Physical activity in periods of sleep deprivation significantly altered the p44/42 MAPK pathway activation (Antle *et al.,* 2008). However, time of day-related expression in other brain areas and its correlation with a phase of neuronal activity in various central nervous system subregions still need to be clarified.

In humans, the connectivity of the amygdala undergoes significant changes between young and middle-aged

individuals (Xiao *et al.*, 2018). In addition, several proteins involved in neuronal plasticity in the basolateral amygdala of rat undergo significant changes during development from postnatal stages till early adulthood, among which is p44/42-MAPK and its target p-CREB (Bessières et al., 2019). p44/42-MAPK expression in the cortex of aged rats is decreased as compared to adult and middle-aged rats (Zhen et al., 1999). Taking all these findings together, expression of p44/42-MAPK exhibit age-related changes in brain structures, consistent with our data that showed that aging decreased p42/44-MAPK expression in the amygdala.

In conclusion, we show a strong correlation between NeuN and p42/44-MAPK expression levels with the circadian system. From young to middle-aged mice, the expression levels of these neuronal proteins in the amygdala decrease, suggesting a strong interaction between the circadian system and aging in the regulation of the neuronal functional state in the amygdala. However, further functional analysis of the electrophysiological properties of amygdalar neurons in addition to behavioral tests is required to confirm this interaction in future studies. Our data may help better understand the role of circadian timing and the effect of age on the neuronal functions within the amygdala and related behavioral changes such as fear and anxiety in health and disease.

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ARABIC SUMMARY

التغيرات المرتبطة بالنظام اليومي وتأثير العمر على اظهار البروتين النووي العصبي وبروتينات المرونه العصبية المسماة بالبروتينات الفسفورية ميتوجين كيناز المنشطة في مجمع اللوزة المخية في الفئران

اميرة امين حسن على [1-2] واحمد النبوى احمد نصر [1]

1- قسم التشريح الادمى و علم الاجنة بكلية الطب جامعه المنصورة مصر

2- قسم التشريح بكليه الطب جامعة دوسيلدورف المانيا

اللوزة المخية هي منطقة دماغية أساسية مسؤولة عن السلوكيات الاجتماعية والقلق. نواة اللوزة الجانبيه، نواة اللوزة القاعدية ونواة اللوزة الإنسي هي نوى مهمه في مجمع اللوزة المخية. هناك تغيير في الاستجابات العاطفية مثل القلق و الخوف على مدار اليوم وكذلك أثناء عملية التقدم فى العمر مما يشير إلى تنظيم وظيفة اللوزة المخية عبر الساعة البيولوجية ، والتي تتأثر أيضًا بالشيخوخة. ولذلك ، قمنا بدراسة التغيرات التى تحدث خلال اليوم بين بداية مرحلة التعرض للضؤ وبدايه فترة الظلمة من اليوم بالإضافة إلى التغيرات المرتبطة بالعمر بين الفئران الصغيرة ومتوسطة العمر و تاثيرها على اظهار البروتين النووي العصبي وبروتينات المرونه العصبية المسماة بالبروتينات الفسفورية ميتوجين كيناز المنشطة في المناطق الفرعية من اللوزة المخية باستخدام صبغات الكيمياء المناعية

تظهر نتائج هذاالبحث ان مستويات الاظهار لـهذه البروتينات تكون اعلى خلال مرحلة الضوء مما يشير إلى وجود علاقة قوية بين النشاط العصبي في اللوزة المخيه والنظام اليومي الذى يشمل فترة اضاءة وفترة اظلام. بالاضافة لذلك تنخفض مستويات التعبير عن هذه البروتينات العصبية في اللوزة فى الفئران خلال منتصف العمر عن الفئران الصغيرة ، مما يشير إلى تفاعل واضح بين النظام اليومي والشيخوخة في تنظيم الحالة الوظيفية العصبية في اللوزة المخية. قد تساعد نتائج هذا البحث في فهم دور التوقيت اليومي وتأثير العمر على الوظائف العصبية داخل اللوزة والتغيرات السلوكية ذات الصلة مثل الخوف والقلق في الحالة الطبيعية وفى الحالات المرضيه