Correlations among central serotonergic parameters and age-related emotional and cognitive changes assessed through the elevated T-maze and the Morris water maze

Luciana Oliveira · Frederico G. Graeff ·
Silvia R. C. Pereira · Ieda F. Oliveira-Silva ·
Glaura C. Franco · Angela Maria Ribeiro

Received: 3 June 2009 / Accepted: 30 November 2009 / Published online: 13 January 2010
© American Aging Association 2010

Abstract Emotion and spatial cognitive aspects were assessed in adult and middle-aged rats using the elevated T-maze (ETM) and the Morris water maze (MWM) tasks. Both adult and middle-aged rats were able to acquire inhibitory avoidance behaviour, though the middle-aged subjects showed larger latencies along the trials, including the baseline, which was significantly longer than that showed by adult rats. Further, compared to adult rats, middle-aged rats had longer escape latency. In spite of the worse performance in the second session of the spatial cognitive task, the middle-aged rats were able to learn the task and remember the information along the whole probe trial test. Both thalamic serotonin (5-HT) concentration and amygdala serotonergic activity (5-HIAA/5-HT) are significantly correlated, respectively,

to escape latency and behavioural extinction in the MWM only for middle-aged rats. A significant correlation between the 5-HIAA/5-HT ratio in the amygdala and behavioural extinction for middle-aged, but not for adult, rats was observed. This result suggests that serotonergic activity in the amygdala may regulate behavioural flexibility in aged animals. In addition, a significant negative correlation was found between hippocampal 5-HIAA/5-HT ratio and the path length at the second training session of the MWM task, although only for adult subjects. This was the only session where a significant difference between the performance of middle-aged and adult rats has occurred. Although the involvement of the hippocampus in learning and memory is well established, the present work shows, for the first time, a correlation between a serotonergic hippocampal parameter and performance of a spatial task, which is lost with ageing.

L. Oliveira · S. R. C. Pereira · I. F. Oliveira-Silva ·
A. M. Ribeiro (✉)
Laboratório de Neurociências Comportamental e
Molecular, LaNeC, Universidade Federal de Minas Gerais,
Belo Horizonte 31270-901, Brazil
e-mail: angelamr@icb.ufmg.br

G. C. Franco
Laboratório de Estatística,
Universidade Federal de Minas Gerais,
Belo Horizonte 31270-901, Brazil

F. G. Graeff
Departamento de Neurociências e Ciências
Comportamentais, FMRP-USP,
Ribeirão Preto 14049-900, Brazil

Keywords Serotonergic parameters · Emotion ·
Cognition · Elevated T-maze · Morris water maze ·
Ageing · Rats

Introduction

Aged subjects often have emotional and motivational impairments (Mattison et al. 2005; Mulligan and Moss 1991) that can be associated to alterations of serotonergic parameters (Kumar et al. 1998). There is also reported evidence pointing to an association

between changes in serotonergic parameters and memory deficits induced by ageing (Da Silva Costa et al. 2009; Perez-Garcia and Meneses 2008). Furthermore, Tohda et al. (2009) have reported evidence indicating that there is a common molecular component involved in both anxiety and memory modulation. Therefore, one possibility to be considered is that at least some kinds of cognitive deficits found in aged subjects could be secondary to emotional dysfunction.

The results of a previous study performed in our laboratory have shown that middle-aged rats can succeed in learning a spatial cognitive task, albeit with significantly worse performance than adult animals. In addition, an age-dependent decrease in serotonin metabolite (5-HIAA) levels was observed in the hippocampus and dorsal raphe nucleus of these animals (Oliveira-Silva et al. 2007). The behavioural test used to assess the spatial cognitive aspects in that study and in many others (Li et al. 2009; Nakajima et al. 2009; Topic et al. 2005) was the Morris water maze (MWM), which is a task that can be considered aversive. One question that can be raised is whether an emotional component could, to some extension, explain the cognitive deficit observed. Thus, in cases of spatial learning and memory studies, it is relevant to include emotional assessment. The elevated T-maze (ETM) is an animal model designed to generate two types of defensive reactions in the same animal, inhibitory avoidance and one-way escape, which have been respectively related to generalised anxiety and panic disorder (Graeff et al. 1993; Pinheiro et al. 2007; Viana et al. 1994) and have been used to assess emotional behaviour.

Central serotonergic systems are thought to be involved in emotional regulation (Cools et al. 2008), and age-related emotional changes have been associated to this system dysfunction (Li et al. 1995). There are interconnections between the neocortex and the hippocampus through the thalamus (Bokor et al. 2002), and these three brain structures are innervated by 5-HT-containing fibres. Studies with old rats have shown morphological alterations in 5-HT-containing fibres of the prefrontal and frontoparietal cortex, striatum, thalamus, hypothalamus, hippocampus, midbrain and spinal cord (Behan and Brownfield 1999; Meltzer et al. 1998). To our knowledge, however, correlations among age-related behavioural changes and alterations in serotonergic parameters in brain regions involved in

both emotion and cognition has not yet been investigated. Hence, the purpose of the present study was to investigate (1) emotional and cognitive measures, assessed in the ETM and MWM, respectively; (2) serotonergic parameters measured in prefrontal cortex, amygdala, thalamus and hippocampus; and (3) correlations among the above behavioural and biochemical parameters. Each one of the biochemical parameters (5-HT and 5-HIAA) was measured and the 5-HIAA/5-HT ratio calculated in order to know whether 5-HT synthesis and/or degradation were affected. The 5-HIAA/5HT ratio indicates 5-HT turnover rate and/or 5-HIAA efflux from the brain.

Materials and methods

Animals and experimental design

Twenty-two male Wistar rats were divided into two groups ($n=11$, each) aged 3 (adults) and 18 months (middle-aged), respectively. All animals were maintained in groups of four or five per cage under a 12-h light/12-h dark cycle, at a temperature of $25\pm 1^\circ\text{C}$, with food and water available ad libitum. Care and use of animals were according to the National Institute of Health Guide for Care and Use of Laboratory Animals. All animals were submitted to ETM and MWM procedures as described below. The animals were killed by decapitation on the first day following the end of behavioural testing. Brain samples were collected, as detailed below, and kept frozen until the day of biochemistry assays.

Behavioural study

Elevated T-maze

Apparatus The ETM was made of wood and had three arms of equal dimensions (50×12 cm). One arm, enclosed by 40-cm high walls, was perpendicular to two opposed open arms. To avoid falls, the open arms were surrounded by a 1-cm high wooden rim. The whole apparatus was elevated 50 cm above the floor.

Procedure Prior to the experimental session, the experimenter gently handled the animal for three consecutive days (5 min per day). One day before the test, the rat was exposed to one of the open arms of

the ETM for 30 min. A wooden barrier mounted on the border of the open arm, between the maze's central area and the arm's proximal end, isolated this arm from the rest of the maze. On the test day, the animal was placed at the end of the enclosed arm, and the time taken to withdraw from this arm with four paws was recorded (baseline latency). The same measurement was repeated twice at 60-s intervals (avoidance 1 and avoidance 2). Following avoidance training (60 s), the rat was placed at the end of the open arm, and the time taken to leave the arm with the four paws out of the arm was recorded (escape latency). The escape latency was evaluated in the same arm to which the rat had been previously exposed. During the 60-s intervals between each trial, the animals were placed in the Plexiglas cage. A cutoff time of 300 s was established for avoidance and escape latencies. Adult and middle-aged rats were submitted to a prior exposure to one of the open arms of the ETM, since previously reported results have shown that this procedure decreases the latency of the first escape response, without affecting inhibitory avoidance, and renders the avoidance task more sensitive to drug effects, in particular, chronic treatment with antidepressants (Pinheiro et al. 2007; Teixeira et al. 2000), probably, because it avoids the interference of novelty.

Morris water maze

Apparatus The apparatus consisted of a 1.80-m diameter fibreglass round pool, filled with water at the depth of 27.5 cm and controlled temperature of $26 \pm 2^\circ\text{C}$. A 15-cm diameter transparent Plexiglas round platform was placed 2 cm below the water surface in the centre of one of the pool quadrants (southeast, southwest, northeast or northwest). In order to avoid visual location of the platform, 60 g of powder milk were dissolved in the water, making it turbid. The pool was placed in a 3×3 -m room, with extra-maze cues (e.g. posters, TV and video equipment, table). All sessions were recorded on VHS with a video camera fixed to the ceiling and connected to a computer. The collection of behavioural data was done automatically, through a program of image analysis developed at the Federal University of Minas Gerais.

Procedure Each animal had one daily session for five consecutive days, and each session consisted of four trials. At the start of each trial, animals were put in the

pool, facing its wall at each of the four quadrant edges, alternated in a pseudo-random fashion. The platform was located in the southeast quadrant in all sessions. The subject was allowed to swim around the pool until it found the platform. If the animal did not find the platform in 60 s, it was gently guided to it by the experimenter. Once on the platform, the animals were allowed to stay there for 20 s. The latency and the path length for finding the platform were recorded. Swimming speed was used as a parameter to evaluate motility.

Probe trial On the sixth day of the reference memory test, each animal was left in the pool without the platform for 120 s. The average distance from the platform during the first (d1) and the second (d2) minutes of probe trial was measured. An extinction index (EI) was calculated by dividing d2 by d1 ($\text{EI} = \text{d2}/\text{d1}$). If d2 is larger than d1, EI will be greater than 1, suggesting behavioural extinction, as the rat was more distant from the previous platform location in the second minute than in the first.

Biochemistry study

Biological sample processing

The orbitofrontal cortex, amygdala, hippocampus and thalamus were quickly dissected out from both hemispheres, and 20 mg of tissue were transferred to a Potter tube containing 500 μL of the following ice-cooled solution: 980 μL 0.1 M perchloric acid, 10 μL 0.4 mM sodium metabisulphite and 10 μL 0.1 mM ethylenediaminetetraacetic acid (EDTA). Tissues were then homogenised and centrifuged for 10 min at $5,300 \times g$. Supernatants were kept to -70°C during 7 days, until their analysis. 5-HT and 5-HIAA contents were measured by high-performance liquid chromatography (HPLC). Two hundred microlitres of the supernatants were injected into the HPLC system for analysis. All procedures were done under the temperature of 0 – 4°C .

Serotonin and 5-hydroxy-indole-acetic acid assay

The HPLC system consisted of a Shimadzu chromatograph (LC-10AD, Tokyo, Japan) with a 200- and 20- μL loop (Rheodyne 7725-I, Cotati, CA, USA) and an electrochemical detector with a glass-carbon electrode (Shimadzu electrochemical detector L-

ECD-6A-Tokyo, Japan), coupled to a LC-10 ad PUMP. The system was equipped with a 3- μ m particle size (150 $\times$ 4.6 mm, ID) C₁₈ analytical column (Hibar-Futigsanle RT) and a pre-packed column (RT 250-4 E. Merck, Darmstadt E.R., Germany). An integrator (Shimadzu C-R7Ae plus) was used to analyse the chromatographic data. For a final volume of 500 mL, the mobile phase consisted of 0.199 M citric acid, 0.243 M sodium acetate, 2.95×10^{-4} M EDTA and methanol (91:9, v/v) adjusted to pH 4.0. The flow-rate was 1.0 mL/min, while the potential was set at 0.85 V. 5-HT and 5-HIAA concentrations were calculated by interpolation of its respective standard curves. Obtained values are reported in nanogrammes per gramme of tissue. The 5-HIAA/5-HT ratio was used as an index of serotonergic system activity.

Statistical analysis

Two-factor analysis of variance (ANOVA; factors: age and session) with repeated measures on the last element was used to assess the behavioural data (avoidance latency in the ETM and the latency and path length during training in the MWM). When a significant interaction between the two factors was found, the average performances of middle-aged and adult groups were separately compared for each session, using the Student's *t* test. The other behavioural (escape latency in ETM, probe trial in MWM) and biochemical parameters data were analysed using the Student's *t* test. Linear regression was used in order to determine the degree to which biochemical and behavioural parameters were correlated. The significant level for all analyses was set at $p < 0.05$.

Results

Behavioural data

Elevated T-maze

ANOVA 2×3 analysis of the behavioural data obtained in the ETM task showed that there was a significant main effect of time ($F_{(2,40)}=4.43$, $p=0.018$), but neither a significant main effect of age ($F_{(1,20)}=3.05$, $p=0.096$) nor a significant interaction between age and time ($F_{(2,20)}=0.25$, $p=0.80$). As can

be seen in Fig. 1a, animals from both groups, adult and middle-aged, were able to learn the task. There was no difference in the speed of acquisition of inhibitory avoidance. The greater latencies showed by the middle-aged animals could be detected at the beginning (base line) of the training.

In Fig. 1b, one can see that the middle-aged animals had a significantly greater escape latency compared to the adult ones ($t=2.25$, $p=0.04$).

Morris water maze

Figure 2a, shows middle-aged and adult animals' latencies in the acquisition sessions of the spatial navigation task. ANOVA 2×5 showed significant interaction between age and sessions ($F_{(4,40)}=4.64$, $p=0.0036$). A post hoc test indicated a significant difference between the groups at the second training session ($t=5.57$, $p<0.001$). Similar results were obtained when the performance of animals was expressed as path length (Fig. 2b). A significant ($F_{(4,40)}=3.98$, $p=0.0082$) interaction between the two factors was found. A post hoc test indicated a significant difference between the groups at the second

Fig. 1 Behavioural data obtained in the elevated T-maze task for adult and middle-aged rats. **a** Acquisition of inhibitory avoidance. **b** Escape latency. There was no difference in the speed of acquisition of inhibitory avoidance. The middle-aged animals had a significantly greater escape latency compared to the adult ones ($t=2.25$, $p=0.04$)

Fig. 2 The middle-aged and adult animals' latencies (a), path length (b) and speed (c) in the acquisition sessions of the spatial navigation task

training session ($t=5.03$, $p<0.001$). At the last session, there was no significant difference ($t=1.25$, $p=0.23$ for latency; $t=0.85$, $p=0.40$ for path length) between the groups. There was no significant main effect (age ($F_{(1,10)}=0.34$, $p=0.57$) and sessions ($F_{(4,40)}=1.43$, $p=0.24$)) nor interaction ($F_{(4,40)}=1.32$, $p=0.28$) in the swimming speed (Fig. 2c).

Probe trial and extinction (data not shown) There were no significant differences between the performance of animals from the two groups during the first minute of probe trial (reference memory; $t=0.68$, $p=0.50$) and between the averages of EI ($t=0.17$, $p=0.87$).

Biochemical data and correlation analysis

Figure 3a–c shows the biochemical data concerning 5-HT and 5-HIAA concentration and the [5-HIAA]/[5-HT] ratio, respectively. These parameters were measured in four regions of central nervous system, namely, prefrontal cortex, hippocampus, thalamus and amygdala. When the data for 5-HIAA content measured in the amygdala obtained from middle-aged (5-HIAA=800±145 ng/g) and adult (5-HIAA=1003±245 ng/g) animals are compared, a tendency to a decrease ($p=0.055$) was found in middle-aged subjects. As for the remaining parameters, no significant differences between middle-aged and adult rats were found.

Fig. 3 [5-HT], [5-HIAA] and the [5-HIAA]/[5-HT] ratio are shown in panels (a), (b) and (c), respectively, for the four regions of central nervous system: prefrontal cortex, hippocampus, thalamus and amygdala

Analyses of regression between behavioural and biochemical parameters were carried out for both middle-aged and adult rats. Scatter-plot diagrams are shown in Fig. 4, and the following significant correlations were found: (1) panel a: between the thalamus 5-HT content and the escape latency ($r=$

0.56, $p=0.006$). When the correlations are calculated separately for middle-aged and adult rats, a significant correlation for middle-aged ($r=0.65$, $p=0.03$), but not for adult ($r=0.43$, $p=0.19$), rats is found, indicating that the higher thalamic the 5-HT content, the better the performance is for middle-aged rats in avoidance

Fig. 4 Scatter-plot diagrams are shown in (a): between the thalamus 5-HT content and the escape latency, (b): between [5-HIAA]/[5-HT] ratio in the hippocampus and performance in the second session during the acquisition of spatial navigation task, and (c): between amygdala [5-HIAA]/[5HT] ratio and extinction index. Correlations were calculated separately for the middle-aged and adults. Details are described in the “Results” section

task; (2) panel b: between [5-HIAA]/[5-HT] ratio in the hippocampus and performance in the second session during the acquisition of spatial navigation task ($r=-0.63$, $p=0.007$). Animal performance is expressed as path length, and the longer the path length, the worse the performance. When the correlations are calculated separately for middle-aged and adult rats, a significant correlation for the adult ($r=-0.84$, $p=0.009$), but not for the middle-aged ($r=-0.25$, $p=0.51$), rats is found, indicating that for adult rats, the higher the hippocampal serotonergic activity, the better the animal's performance is in the beginning of acquisition process; and (3) panel c: between amygdala [5-HIAA]/[5HT] ratio and EI ($r=0.40$, $p=0.010$). In this case, when the correlations are calculated separately, a significant correlation for the middle-aged ($r=0.77$, $p=0.04$), but not for adults ($r=0.41$, $p=0.31$), is found. This indicates that for middle-aged animals, the higher the amygdala serotonergic activity, the better is the behavioural flexibility.

Discussion

The acquisition of inhibitory avoidance in the ETM is expressed by the gradual increase of the latency to leave the closed arm (Graeff et al. 1993; Viana et al. 1994). The present results show that both adult and middle-aged rats were able to acquire this behaviour, though the middle-aged subjects showed larger latencies along the trials, including the baseline, which is significantly longer than in adult rats ($p=0.046$, one-tailed). Several hypotheses can be raised to explain this result: (1) the occurrence of a motor impairment that could interfere with ambulation, (2) an anxiogenic effect of ageing, and (3) less motivation shown by the middle-aged rats. The first hypothesis seems unlikely, since middle-aged rats showed swimming speed similar to that of adult rats in the MWM. However, one could argue that compared to the ETM, the aversive motivation in the MWM is stronger, given that the animal has to swim to stay alive. Another argument against the first hypothesis is that in a previous study carried out in the same laboratory, comparison between adult and middle-aged groups showed no significant differences in rota-rod performance (Oliveira-Silva et al. 2007). However, considering that rota-rod performance depends on motor coordination and equilibrium, dysfunction of other

motor functions cannot be discarded. Regarding the second hypothesis, the influence of anxiety can be discarded by the evidence that there was no difference between middle-aged and adult performance in the acquisition of inhibitory avoidance: as shown in Fig. 1a, the slope of the avoidance acquisition line is similar for the two groups. Thus, the third motivational assumption seems to be the most likely, since the longer latency shown by middle-aged rats in the baseline trial can be due to lack of motivation to explore. Indeed, at the baseline, the subjects do not know that there will be an aversive situation ahead and, so, they are exploring a new environment, rather than performing inhibitory avoidance. In agreement, age-related decline in motivation has been reported (Mattison et al. 2005) and, in particular, there is evidence showing that specific changes in sensory and regulatory systems are likely to result in waning motivation and mood during ageing (Mulligan and Moss 1991). Nevertheless, further studies are necessary to clarify this question.

The present results with the ETM further show that middle-aged rats had longer escape latency compared to adult ones. Alone, this result would indicate impaired escape, usually interpreted as lessened panic-related fear (Pinheiro et al. 2007). However, having in mind that exploratory behaviour is reduced, as indicated by the longer baseline latency in the avoidance task, the most parsimonious explanation is that the longer escape latency shown by middle-aged rats is also due to a general decrease in motivation and/or motor ability, as previously discussed. Other possibilities to be considered are impairment in defensive behaviour (e.g. escape from a dangerous situation) and/or decreased capacity of assessing risk (e.g. unable to give the right value to environmental threats). Hunt et al. (2009) showed that aged rats are less reactive than younger rats to predator odours due to decreased responsiveness in defence-related but not necessarily olfactory circuits. Another matter to be considered is that impairment in processes responsible for “decision making” and/or “planning” could interfere with escape performance. Recently, Kennedy and Raz (2009) have shown that age-related degeneration in forebrain areas is associated with decreased processing speed and poor working memory. Similarly, Head et al. (2009) have found that age-related change in perseveration is completely accounted for by a decline in processing speed and temporal processing, which

are deficits of working memory associated with decreased prefrontal cortical volume. All of that could interfere with the escape response.

Compared to adult rats, middle-aged animals showed different speed of acquisition of a spatial cognitive task. In spite of the worse performance in the second session, the middle-aged rats were able to learn the task and remember the information along the whole probe trial. Regarding behavioural extinction, their performance was also similar to that of adults. The results of the acquisition task are similar to those previously obtained by our group (Oliveira-Silva et al. 2007), except that in these earlier reported results, middle-aged rats have shown worse memory performance during the probe trial and a lower EI. All serotonergic parameters assessed were not changed by ageing in any of the studied brain area (neocortex, hippocampus, thalamus and amygdala), also not in accordance with the results reported by Oliveira-Silva et al. (2007), who found an ageing effect on hippocampal 5-HIAA level. One possible explanation for these inconsistencies is the differences in experimental design. For instance, in the present study, the rats were housed in groups of five per cage, whereas in the previous study, there was only one rat per cage. The latter condition of social separation, which is known to affect emotional behaviour as well as the brain metabolism of the 5-HT precursor tryptophan (Miura et al. 2008), may have influenced the cognitive and biochemical parameters assessed. In further studies, control of social isolation is advisable to clarify this question. Regarding the ageing effect on central serotonergic parameters, it is worth remarking that there are several inconsistencies among the data reported by different research groups (McEntee and Crook 1991; Meltzer et al. 1998; Menezes et al. 2004).

We have observed a nearly significant trend ($p=0.055$) for a significant main effect of age on 5-HIAA concentration in the amygdala, aged animals showing a decrease, compared to adult ones. The amygdala is a limbic structure located in the anterior medial temporal lobe that is known to play a pivotal role in emotional learning and memory (Weike et al. 2005; Yang et al. 2008). Lee et al. (2001), measuring 5-HIAA in the neocortex, found similar results for the effect of ageing. These results could be explained by the following factors: (1) increase in active transport of the 5-HIAA out of the brain and/or (2) increase in

levels of the cytoplasmic serotonin binding protein (SBP) and/or dysfunction in the mechanism of 5-HT release from SBP, which could lead to a decreased access of 5-HT to the catabolic enzyme monoamine oxidase (MAO), and/or (3) a decreased MAO activity. However, it is known that MAO activity and content increase with ageing in mammalian species (Nicotra et al. 2004). The first hypothesis is in accordance with the evidence that age increases brain barrier permeability (Bartels et al. 2009). Regarding the second hypothesis, as far as we know, there is no published data on the effect of ageing on SBP content and/or function. This protein is related to 5-HT storage or its transport and protection from MAO degradation (Del Rio et al. 1995). If ageing affects SBP, it could increase cytoplasmic 5-HT. However, no significant change in the level of this neurotransmitter was observed in the middle-aged group, which may indicate activation of a feedback mechanism regulating 5-HT biosynthesis, since no significant difference between adult and middle-aged group in the amygdala 5-HIAA/5-HT ratio was found. Through this putative mechanism, the level of the 5-HT would be adjusted to physiological range in order to maintain neurotransmission effectiveness.

It is interesting to remark that data obtained in the present study indicate that both thalamic 5-HT concentration and amygdala serotonergic activity are significantly correlated to escape latency and EI, respectively, but only for middle-aged rats. In this regard, there is a wealth of reported evidence about the involvement of the serotonergic system in emotional regulation (Cools et al. 2008). Nevertheless, as far as we know, an association between serotonergic parameters and these specific behavioural parameters has not yet been reported. In addition, a significant correlation between the 5-HIAA/5-HT ratio in the amygdala and behavioural extinction in the MWM for middle-aged, but not for adult, rats was presently observed. This result suggests that serotonergic activity in the amygdala may be associated to behavioural flexibility in aged animal. Maybe some injury caused by ageing could be compensated by a kind of functional adjustment through the serotonergic system to keep extinction performance similar to that of adult subjects. However, the same rationale may not be appropriate for escape latency, which was significantly correlated to thalamic serotonin (5-HT) concentration in middle-aged rats only, since the

escape response of these animals was significantly different from that of adult rats. No correlation between 5-HIAA concentration and any measured behavioural parameter was observed. Further studies are necessary to better understand the full meaning of these data.

It is known that both the neocortex and the hippocampus are affected by ageing, and that these brain areas play a pivotal role in memory (Arendt et al. 1989; Pires et al. 2005). A significant negative correlation was presently found between hippocampal 5-HIAA/5-HT ratio and the path length at the second training session of the MWM task, although only for adult subjects. This was the only session where a significant difference between the performance of middle-aged and adult rats has occurred. This result suggests that the ageing process changes the hippocampal serotonergic system, interfering with its role in regulating specific behavioural processes. In this way, the present result showing that middle-aged rats perform less well than adult ones in the spatial navigation task may be viewed as indicating hippocampal serotonergic system participation in the initial acquisition process.

In short, both adult and middle-aged rats were able to acquire inhibitory avoidance behaviour, though the middle-aged subjects showed longer latencies along the trials, including the baseline. Further, compared to adult rats, middle-aged rats had longer escape latencies. In spite of the worse performance in the second session of the spatial cognitive task, the middle-aged rats were able to learn the task. Both thalamic 5-HT concentration and amygdala 5-HIAA/5-HT ratio are correlated, respectively, to escape latency and behavioural extinction in the MWM, but only for middle-aged rats. Furthermore, a correlation between the 5-HIAA/5-HT ratio in the amygdala and behavioural extinction for middle-aged, but not for adult, rats was observed. Hence, the serotonergic activity in the amygdala may regulate behavioural flexibility in aged animals. In addition, a correlation was found between hippocampal 5-HIAA/5-HT ratio and the path length at the second training session of the MWM task only for adult subjects. Therefore, hippocampal serotonergic activity may participate in the performance of spatial tasks. Although the involvement of the hippocampus in learning and memory is well established (Squire 1993), the present work shows, for the first time, a correlation between a

serotonergic hippocampal parameter and performance of a spatial task, which is lost with ageing.

Acknowledgements This work was supported by CNPq (Conselho Nacional do Desenvolvimento da Pesquisa) and FAPEMG (Fundação de Amparo a Pesquisa de Minas Gerais). Aparecida Guerra de Jesus received scholarship from FAPEMIG for technical assistance. Frederico G. Graeff was supported by fellowship grants from FAEPA (Fundação de Amparo à Pesquisa, Ensino e Assistência do Hospital da Clínicas da Faculdade de Medicina de Ribeirão Preto, USP) and from the Brazilian CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). The authors thank to Vitor Purri, undergraduate student of Engineering at Universidade Federal de Minas Gerais, for the development of the program of image analysis used in this study.

References

- Arendt T, Allen Y, Marchbanks RM, Schugens MM, Sinden J, Lantos PL, Gray JA (1989) Cholinergic system and memory in the rat: effects of chronic ethanol, embryonic basal forebrain brain transplants and excitotoxic lesions of cholinergic basal forebrain projection system. *Neuroscience* 33:435–462
- Bartels AL, Kortekaas R, Bart J, Willemsen AT, de Klerk OL, de Vries JJ, van Oostrom JC, Leenders KL (2009) Blood-brain barrier P-glycoprotein function decreases in specific brain regions with aging: a possible role in progressive neurodegeneration. *Neurobiol Aging* 30:1818–1824
- Behan M, Brownfield MS (1999) Age-related changes in serotonin in the hypoglossal nucleus of rat: implications for sleep-disordered breathing. *Neurosci Lett* 267:133–136
- Bokor H, Csaki A, Kocsis K, Kiss J (2002) Cellular architecture of the nucleus reuniens thalami and its putative aspartatergic/glutamatergic projection to the hippocampus and medial septum in the rat. *Eur J Neurosci* 16:1227–1239
- Cools R, Roberts AC, Robbins TW (2008) Serotonergic regulation of emotional and behavioural control processes. *Trends Cogn Sci* 12:31–40
- Da Silva Costa V, Duchatelle P, Boulouard M, Dauphin F (2009) Selective 5-HT₆ receptor blockade improves spatial recognition memory and reverses age-related deficits in spatial recognition memory in the mouse. *Neuropsychopharmacol* 34:488–500
- Del Rio MJ, Velez-Pardo C, Ebinger G, Vauquelin G (1995) Serotonin binding proteins “SBP”: target proteins and tool for in vitro neurotoxicity studies. *Gen Pharmacol* 26:1633–1641
- Graeff FG, Viana MB, Tomaz C (1993) The elevated T-Maze, a new experimental-model of anxiety and memory - effect of diazepam. *Braz J Med Biol Res* 26:67–70
- Head D, Kennedy KM, Rodrigue KM, Raz N (2009) Age differences in perseveration: cognitive and neuroanatomical mediators of performance on the Wisconsin Card Sorting Test. *Neuropsychologia* 47:1200–1203

- Hunt GE, Van Nieuwenhuijzen PS, Chan-Ling T, McGregor IS (2009) 'When an old rat smells a cat': a decline in defense-related, but not accessory olfactory, Fos expression in aged rats. *Neurobiology of Aging* (in press)
- Kennedy KM, Raz N (2009) Aging white matter and cognition: differential effects of regional variations in diffusion properties on memory, executive functions, and speed. *Neuropsychologia* 47:916–927
- Kumar AM, Weiss S, Fernandez JB, Cruess D, Eisdorfer C (1998) Peripheral serotonin levels in women: role of aging and ethnicity. *Gerontology* 44:211–216
- Lee JJ, Chang CK, Liu IM, Chi TC, Yu HJ, Cheng JT (2001) Changes in endogenous monoamines in aged rats. *Clin Exp Pharmacol Physiol* 28:285–289
- Li JW, Watanabe M, Fujisawa Y, Shibuya T (1995) Relation between age-related changes in hyper-emotionality and serotonergic neuronal activities in the rat limbic system. *Nihon Shinkei Seishin Yakurigaku Zasshi* 15:231–238
- Li Q, Zhao HF, Zhang ZF, Liu ZG, Pei XR, Wang JB, Cai MY, Li Y (2009) Long-term administration of green tea catechins prevents age-related spatial learning and memory decline in c57bl/6 j mice by regulating hippocampal cyclic amp-response element binding protein signaling cascade. *Neuroscience* 159:1208–1215
- Mattison JA, Black A, Huck J, Moscrip T, Handy A, Tilmont E, Roth GS, Lane MA, Ingram DK (2005) Age-related decline in caloric intake and motivation for food in rhesus monkeys. *Neurobiol Aging* 26:1117–1127
- McEntee WJ, Crook TH (1991) Serotonin, memory, and aging brain. *Psychopharmacol* 103:143–149
- Meltzer CC, Smith G, DeKosky ST, Pollock BG, Mathis CA, Moore RY, Kupfer DJ, Reynolds CF III (1998) Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging. *Neuropsychopharmacol* 18:407–430
- Menezes A, Manuel-Apolinar L, Rocha L, Castillo E, Castillo C (2004) Expression of the 5-HT receptors in rat brain during memory consolidation. *Behav Brain Res* 152:425–436
- Miura H, Ozaki N, Shirokawa T, Isobe K (2008) Changes in brain tryptophan metabolism elicited by ageing, social environment, and psychological stress in mice. *Stress-Int J Biol Stress* 11:160–169
- Mulligan T, Moss CR (1991) Sexuality and aging in male veterans: a cross-sectional study of interest, ability, and activity. *Arch Sex Behav* 20:17–25
- Nakajima S, Ohsawa I, Nagata K, Ohta S, Ohno M, Ijichi T, Mikami T (2009) Oral supplementation with melon superoxide dismutase extract promotes antioxidant defenses in the brain and prevents stress-induced impairment of spatial memory. *Behav Brain Res* 200:15–21
- Nicotra A, Pierucci F, Parvez H, Senatori O (2004) Monoamine oxidase expression during development and aging. *Neurotoxicology* 25:155–165
- Oliveira-Silva IF, Pinto L, Pereira SR, Ferraz VP, Barbosa AJ, Coelho VA, Gualberto FF, Souza VF, Faleiro RR, Franco GC, Ribeiro AM (2007) Age-related deficit in behavioural extinction is counteracted by long-term ethanol consumption: correlation between 5-HIAA/5HT ratio in dorsal raphe nucleus and cognitive parameters. *Behav Brain Res* 180:226–234
- Perez-Garcia G, Meneses A (2008) Memory formation, amnesia, improved memory and reversed amnesia: 5-HT role. *Behav Brain Res* 195:17–29
- Pinheiro SH, Zangrossi H, Del Ben CM, Graeff FG (2007) Elevated mazes as animal models of anxiety: effects of serotonergic agents. *An Acad Bras Ci* 79:71–85
- Pires RG, Pereira SR, Oliveira-Silva IF, Franco GC, Ribeiro AM (2005) Cholinergic parameters and the retrieval of learned and re-learned spatial information: a study using a model of Wernicke-Korsakoff Syndrome. *Behav Brain Res* 162:11–21
- Squire LR (1993) The hippocampus and spatial memory. *Trends Neurosci* 16:56–57
- Teixeira RC, Zangrossi H, Graeff FG (2000) Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. *Pharmacol Biochem Behav* 65:571–576
- Tohda C, Nakanishi R, Kadowaki M (2009) Hyperactivity, memory deficit and anxiety-related behaviors in mice lacking the p85 alpha subunit of phosphoinositide-3 kinase. *Brain Dev* 31:69–74
- Topic B, Dere E, Schulz D, Souza Silva MA, Jocham G, Kart E, Huston JP (2005) Aged and adult rats compared in acquisition and extinction of escape from the water maze: focus on individual differences. *Behav Neurosci* 119:127–144
- Viana MB, Tomaz C, Graeff FG (1994) The elevated T-maze: a new animal model of anxiety and memory. *Pharmacol Biochem Behav* 49:549–554
- Weike AI, Hamm AO, Schupp HT, Runge U, Schroeder HW, Kessler C (2005) Fear conditioning following unilateral temporal lobectomy: dissociation of conditioned startle potentiation and autonomic learning. *J Neurosci* 25:11117–11124
- Yang RJ, Mozhui K, Karlsson RM, Cameron HA, Williams RW, Holmes A (2008) Variation in mouse basolateral amygdala volume is associated with differences in stress reactivity and fear learning. *Neuropsychopharmacol* 33:2595–2604