Pharmacology, Biochemistry and Behavior 95 (2010) 351–358

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The influence of the long-term administration of *Curcuma longa* extract on learning and spatial memory as well as the concentration of brain neurotransmitters and level of plasma corticosterone in aged rats

Justyna Pyrzanowska [a], Agnieszka Piechal [a], Kamilla Blecharz-Klin [a], Małgorzata Lehner [b], Anna Skórzewska [b], Danuta Turzyńska [b], Alicja Sobolewska [b], Adam Plaznik [b], Ewa Widy-Tyszkiewicz [a,*]

[a] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland
[b] Department of Neurochemistry, Institute of Psychiatry and Neurology, Sobieskiego 9, 02-957 Warsaw, Poland

ARTICLE INFO

Article history:
Received 31 March 2009
Received in revised form 13 December 2009
Accepted 21 February 2010
Available online 26 February 2010

Keywords:
Curcuma longa
Curcumin
Water maze
5-Hydroxytryptamine
Taurine
Glutamic acid
Corticosterone
Prefrontal cortex
Hippocampus
Aged rats

ABSTRACT

The effects of chronic pre-treatment with a standardised extract of *Curcuma longa* on learning and spatial memory in aged 24-month old male Wistar rats were estimated in a Morris water maze paradigm. Animals received the extract orally for two months in prepared rodent chow to obtain the doses 10 and 50 mg/kg/day. At the end of behavioural trials the concentration of neurotransmitters, their metabolites and amino acids in selected brain regions were estimated.

There was a significant decrease in escape latency over four days of training in both treated groups in comparison to the control group. In a probe trial on the 5th day the C10 group crossed the target area more often and spent more time in the SE quadrant than control group.

Significant differences in brain monoamines and amino acid levels between groups were noticed. The increase in the 5-HT (5-hydroxytryptamine) level in the prefrontal cortex correlated positively with the number of crossings over the target area during the first probe trial in both pre-treated groups. The plasma corticosterone level was lower in both pre-treated groups than in the control group. This suggests enhanced learning ability and spatial memory after *C. longa* extract treatment with the modulation of central serotoninergic system activity, and may be linked with an increased tolerance to stress conditions.

A decrease in hippocampal glutamate in animals given plant extract compared to control rats was observed. It is possible that extract may influence a reduction in glutamate-induced excitotoxicity and consequently the neurodegeneration processes in the hippocampus.

1. Introduction

Curcuma longa is a well known medicinal plant with established anti-cancer, anti-inflammatory and neuroprotective effects (Goel et al., 2008; Aggarwal and Harikumar, 2009). Its rhizome contains essential oils (such as turmerones and zingiberene), turmerin (a water-soluble peptide) and curcuminoids which can be defined as phenolic compounds derived from the roots (Hiserodt et al., 1996). The most important constituent of the standardised extract of dried rhizome of *C. longa* is curcumin (diferuloylmethane), a low molecular weight polyphenol generally regarded as the most active constituent.

The neuroprotective effects of curcumin seem to be connected with its influence on various intra- and extra-cellular mechanisms in central nervous system that are responsible for antioxidant and anti-inflammatory properties (Yamamoto et al., 1997; Khopde et al., 1999; Lim et al., 2001; Quiles et al., 2002; Hong et al., 2004; Bala et al., 2006; Jacob et al., 2007; Zafir and Banu, 2007), the inhibition of beta-amyloid formation (Frautschy et al., 2001; Yang et al., 2005; Garcia-Alloza et al., 2007), and the reversion of stress-induced impairment of neurogenesis (Xu et al., 2007), and anti-proliferative and proapoptotic effects (Sandur et al., 2007). Curcumin also stops lipid peroxidation in animal brains (Ramirez-Tortosa et al., 1999) and protects against heavy-metal neurotoxicity (Daniel et al., 2004; Maheshwari et al., 2006), glutamate excitotoxicity (Matteucci et al., 2005; Suh et al., 2007), ischemia (Ghoneim et al., 2002; Jiang et al., 2007; Zhao et al., 2008) and hyperglycaemia (Kuhad and Chopra, 2007). Recently Conboy et al. (2009) noticed, that curcumin, given into the stomach by gavage, at the dose of 75 mg for 8 days, improves learning and memory in 18 month old Wistar rats in the water maze.

Some research has shown that curcumin may influence neurotransmission in CNS and alterations of behaviour in animal models of depression (Xu et al., 2007; Xia et al., 2007; Bishnoi et al., 2008; Kulkarni et al., 2008; Bhutani et al., 2009; Li et al., 2009).

* Corresponding author. Tel./fax: +48 22 826 21 16.
E-mail address: etyszkiewicz@wum.edu.pl (E. Widy-Tyszkiewicz).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.02.013

This study is concerned with the influence of the long-term pre-treatment of a standardised extract of *C. longa* on spatial memory in a water maze, and presents alterations in brain neurotransmitters and amino acids, as well as plasma corticosterone levels in naturally aged rats.

2. Methods and materials

2.1. Animals

The effect of long-term administering a standardised extract of the *C. longa* rhizome was analysed in a modified water maze task in 24-month old male Wistar rats (after 2 months of plant extract pre-treatment). The body weight of the animals did not differ between control and experimental groups ($F_{(2,20)}=0.32$, $p<0.73$ and $F_{(2,20)}=1.11$, $p<0.35$ at the beginning and at the end of the experiment respectively) and did not change significantly within 2 months duration of extract intake.

The rats were housed in typical plastic cages, in the special animal room under a 12:12 h light: dark cycle with free access to the water. The rats were acclimatised to laboratory conditions before the behavioural test. All the procedures took place between 8 a.m. up to 4 p.m., and each rat was used only one time in the water maze.

All animal testing was carried out according to the European Communities Council Directive (86/609/EEC) of 24 November 1986, after approval of the Ethical Committee for Animal Experiments at the Medical University of Warsaw.

2.2. Curcumin extract treatment schedule

The standardised extract of *C. longa* rhizome — CPE-014 (Arjuna Natural Extracts Ltd; India) was a gift from Labofarm. The content of the active ingredients curcuminoids (curcumin I, curcumin II — demethoxycurcumin and curcumin III — bisdemethoxycurcumin) amounted to 95.9%.

The curcumin extract was added directly to the powdered chow, mixed carefully and after slight wetting, the fresh pellets of chow were formed each day by hand. The chow was given to 22 month old rats for 60 days and for the time of behavioural experiment. Each rat received 20 g of chow every day. Such amount of chow had been earlier precisely estimated in pilot trials as the amount is completely eaten and sufficient. The control of feeding and rat's weight was continuously checked within experiment. The control rats were given standardised laboratory chow and the treated ones the same chow with addition of the extract using the concentrations of 350 and 1750 ppm. The daily amounts of plant extract used during the whole experiment provided the doses between 9.5 and 10.3 mg/kg (mean value 9.99, SD = 0.28) and 48.0–51.5 mg/kg (mean value 49.59, SD = 1.33) in the C10 and C50 group respectively.

The animals were randomised, three groups of rats were established and treated as follows: 1) the control group received 20 g of standard chow without curcumin extract each day (Con, $n=7$), and 2) two treatment groups received the *C. longa* extract in 20 g of the prepared chow to provide the doses circa 10 mg/kg b.w. (C10, $n=8$) and 50 mg/kg b.w. (C50, $n=8$) respectively each day.

2.3. Behavioural assessment

2.3.1. Water maze test

The animals were trained in a water maze for four days. Memory was estimated by a trial on the 5th day, given 24 h after the previous training, which consisted of four trials daily. Motivational factors and motor abilities were analysed in the visible platform test. A modified version of the Morris water maze was used (Widy-Tyszkiewicz et al., 1993). A 1.40 m diameter pool filled with 23 °C water was used. The area of the water maze was divided into four quadrants designated NE, NW, SE, and SW. The animals were trained to locate a transparent hidden plexiglass platform placed 1 cm below the water surface in the pool that was surrounded by different cues to spatial coordinates. The animals from each group were given one session of four trials daily for four consecutive days. During every trial the rat was located in the water with its face turned to the wall of the pool at one of three equally spaced starting points. These points were situated in quadrants without the platform and changed in each trial and day. The hidden platform was located in the SE (Southeast) quadrant. A trial was terminated when the rat reached and entered the platform, or after 60 s elapsed. If the animal did not find the platform within this time, it was placed on the platform for 15 s before the next trial was initiated. The first memory test was conducted on the 5th day, 24 h after the previous training. There was no platform in the SE sector during the probe trial when the rats were allowed to swim for 60 s before the end of the session. The visible platform test was carried out on the 6th day. The animals had to find the well-signed platform placed 1 cm over the water surface in four trials from different starting points to the cued target.

Post mortem examination was carried out not less than 24 h after the completion of behavioural testing.

Data from the water maze (latencies to locate the platform, distance travelled, number of crossings in a target area and time spent in the goal quadrant) were recorded by a VHS image analysing system (Chromotrack, San Diego Instruments).

2.4. Biochemistry assessment

2.4.1. Corticosterone

The rats were sacrificed, and 500 µl samples of blood were collected from the body trunk into heparinised tube for a corticosterone assay (Mercier et al., 2003). The samples were immediately centrifuged (2600×g at 4 °C for 15 min). Plasma was extracted and stored at −70 °C, until analysis.

Plasma corticosterone levels were analysed by radioimmunoassay [^{3}H] RIA kit, MP Biomedicals Inc. The assay was performed in duplicate. Plasma was diluted (1:200) with steroid diluent and assayed according to kit instructions. Prior to the assay, all samples were incubated at 98 °C for 10 min, to denature the corticosterone binding proteins in plasma. The corticosterone antibody cross-reactivity with other naturally occurring adrenal steroids was <0.08%, except for desoxycorticosterone (6.10%), progesterone (0.29%) and cortisol (0.19%). The concentration range for this assay was 0.025–1 ng/0.5 ml with a six point calibration curve 0.025; 0.05; 0.1; 0.25; 0.5; 1 ng/0.5 ml. Intra-assay variability was 5%.

2.4.2. Monoamines

The concentrations of monoamines and metabolites in selected brain regions were estimated. Biochemical measurements were carried out 24 h after the last behavioural trial. The rats were decapitated, their brains immediately removed and dissected out on an ice-cold plate according to the method of Glowinski and Iversen (1966), into following regions: prefrontal cortex, hippocampus, hypothalamus and striatum. Each tissue was placed in a dry ice-cooled polyethylene vial, weighed, and stored in a deep freezer at −80 °C until assayed. Then the tissues were homogenised with a polytron-type homogeniser (30 s, 4 °C) in ice-cold 0.2 M perchloric acid with internal standard added DHBA (dihydroxybenzylamine). The homogenates were centrifuged at 26,880×g for 8 min at 4 °C. Then supernatants were collected and filtered through 0.45 µm filters(Millipore) and examined for neurotransmitter content.

The determination of NE, DA, 5-HT and their metabolites was performed using a modified high pressure liquid chromatography (HPLC) method reported by Kaneda et al. (1986). The HPLC system consisted of a Shimadzu LC-10AP VP pump electrochemical detector with a flow-through cell (Decade-Antec Leyden). A high-density glassy carbon-working electrode was operated at +840 mV. The sample was injected manually using Rheodyne 7725i injection valve with a 20 µl sample loop. The separation of monoamines and their metabolites was obtained on the Phenomenex Luna 3 µm C18(2)

100 A (150 mm × 3 mm) with a Phenomenex KJO-4282 precolumn. The column temperature was 32 °C. The mobile phase consisted of 4.1 mM disodium phosphate, 0.027 mM ethylenediaminetetraacetic acid (EDTA), 7.95 mM citric acid, 0.175 M sodium chloride, 0.34 mM octane sulphonic acid and 14% methanol. It was filtrated through 0.45 µm filters (Millipore). The flow rate was 0.4 ml/min. The mobile phase was degassed with helium. Chromatogram registration and analysis were carried out using ChromaX 2004 software. The concentration of monoamines and their metabolites was calculated as ng/ml.

2.4.3. Amino acids

An HPLC analysis of amino acids was performed using a Luna 5 µm C18(2) 100 A (250 mm × 4.6 mm) reverse phase column. Compounds were eluted isocratically with a mobile phase delivered at 0.7 ml/min using a Shimadzu Class VP LC 10AD pump. An electrochemical detector with a flow-through cell (Intro-Antec Leyden), linked to a Shimadzu Class VP Integrator SCL-10Avp, was used. A high-density glassy carbon-working electrode (Antec) was operated at +0.85 V. A Rheodyne injection valve with a 20 µl sample loops was used to manually inject the samples. The preparation of the mobile phase and the derivatising agents were based on the method of Rowley et al. (1995) with some modifications. The mobile phase consisted of 45 mM disodium phosphate and 0.15 mM ethylenediaminetetraacetic acid (EDTA) with 24% methanol (v/v) water adjusted to pH 3.9 with 0.2 M citric acid. It was then filtered through 0.45 µm filters and degassed for 15 min. Stock solutions (0.01 M) of amino acids standards were prepared in double deionised water and kept at 4 °C for five days. To prevent adhesion to the glass, amino acid (especially GABA) standards were prepared in polyethylene vials. Working solutions were prepared daily by dilutions of the stock solution. To obtain agents for derivatisation o-phthaldialdehyde (OPA, 22 mg, Fluka) was dissolved in 0.5 ml of 1 M sodium sulphite, 0.5 l of methanol, and 0.9 ml of sodium tetraborate buffer (0.1 M) adjusted to pH 10.4 with 5 M sodium hydroxide (Rowley et al., 1995). The reaction of derivatisation was performed at room temperature. The derivatising agent (20 µl) was reacted with 1 ml of amino acid standard for 15 min in a polyethylene vial before injection onto the column. For reaction with supernatant samples (20 µl), the volume of the derivatising agent was reduced to 0.4 µl to eliminate the contamination of chromatogram by excessive reagent, which is electroactive. The concentration of amino acids was calculated as µM.

2.5. Statistical analysis

ANOVA with repeated measures (treatment × day × trial) was used to assess differences during the acquisition learning. All post-hoc tests were performed using the Newman–Keuls test to identify any significant differences. Pearson's correlation coefficients r were calculated with simple linear regression analysis based on the results of the probe trials in the water maze and the level of monoamines, their metabolites, neurotransmitters turnover and selected amino acids in all tested brain regions (prefrontal cortex, hippocampus, hypothalamus and striatum). A similar analysis was conducted with the results of learning performance and plasma corticosterone levels. All the hypotheses tested used a significance level of 0.05.

3. Results

3.1. Behaviour — water maze results

3.1.1. Acquisition trials (Days 1–4, trials 1–16)

The results of the training in the water maze test are presented in Fig. 1a, b and Fig. 2a, b.

ANOVA analysis for a particular day of training and escape latency is presented as follows: Day 1: $F_{(2,20)} = 1.49$, $p < 0.25$, Day 2: $F_{(2,20)} = 1.48$, $p < 0.25$, Day 3: $F_{(2,20)} = 3.26$, $p < 0.06$, Day 4: $F_{(2,20)} = 4.79$, $p < 0.02$. The Newman–Keuls analysis showed a decrease of latency in C10 group vs

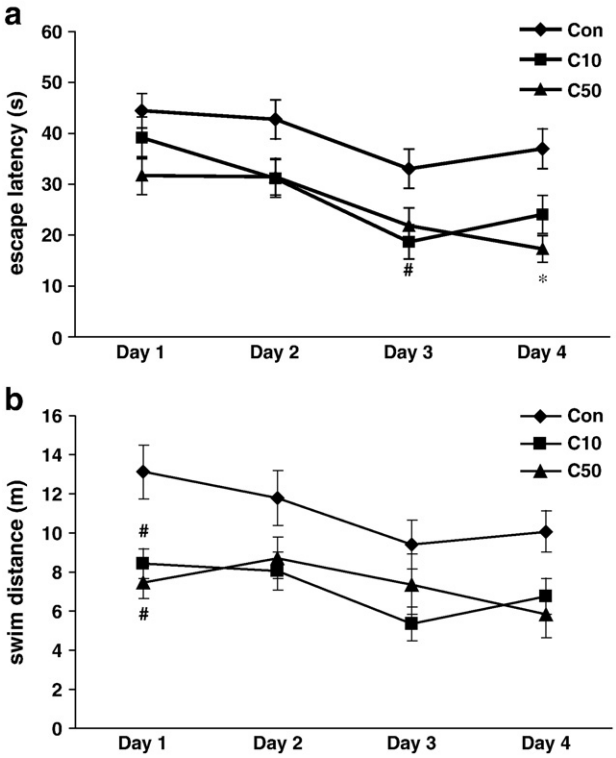

Fig. 1. a. Effects of 60 days pre-treatment with a standardised extract of *Curcuma longa* on learning and the memory of aged male Wistar rats in the water maze - mean escape latency (±SE) during acquisition trials between examined groups on subsequent days, # Day 3: C10 vs control, $p < 0.05$, NK and * Day 4: C50 vs control, $p < 0.05$, NK. b. Effects of 60 days pre-treatment with a standardised extract of Curcuma longa on learning and the memory of aged male Wistar rats in the water maze - mean swim distance (±SE) during acquisition trials between examined groups on subsequent days, # Day 1: C10 vs control, $p < 0.01$, NK , and C50 vs control, $p < 0.01$, NK.

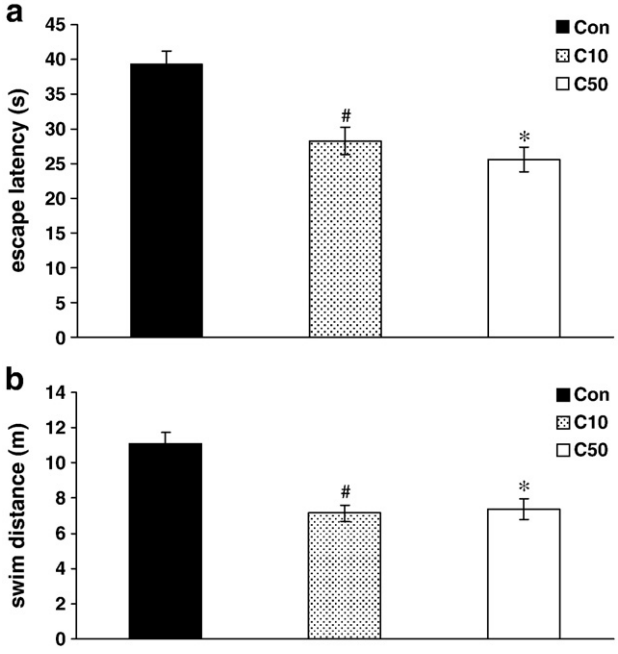

Fig. 2. Effects of 60 day pre-treatment with a standardised extract of *Curcuma longa* on learning and the memory of aged male Wistar rats in the water maze. a. Mean escape latency (±SE) during acquisition trials between examined groups in the course of training, #C10 vs control, $p < 0.05$, NK, *C50 vs control, $p < 0.05$, NK. b. Mean swim distance (±SE) during acquisition trials between examined groups in the course of training, #C10 vs control, $p < 0.05$, NK, *C50 vs control, $p < 0.05$, NK.

control on 3rd day and in C50 vs control on 4th day ($p<0.05$) (Fig. 1a). The values of mean total escape latency during learning (Days 1–4) were significantly different among the groups (Con: 39.31 ± 1.9 s; C10: 28.27 ± 1.97 s; C50: 25.6 ± 1.76 s) ($F_{(2,20)}=4.37$, $p<0.03$). A Newman–Keuls analysis of latencies showed a significant decrease in mean total escape latency for both treatment groups in comparison with controls ($p<0.05$) (Fig. 2a).

ANOVA analysis for a particular day of training and swim distance is presented as follows: Day 1: $F_{(2,20)}=5.97$, $p<0.01$, Day 2: $F_{(2,20)}=1.62$, $p<0.22$, Day 3: $F_{(2,20)}=1.67$, $p<0.21$, Day 4: $F_{(2,20)}=2.42$, $p<0.11$. The Newman–Keuls analysis showed a decrease in mean distance in C10 and C50 groups vs control on 1st day ($p<0.01$) (Fig. 2b). The total swim distance was significantly different between the groups (Con: 11.09 ± 0.65 m; C10: 7.14 ± 0.45 m; C50: 7.37 ± 0.59 m) ($F_{(2,20)}=4.22$, $p<0.03$) (Fig. 2b). A Newman–Keuls analysis of the paths travelled showed a significant decrease in mean swim distance for both treatment groups in comparison with controls ($p<0.05$).

The results did not show a significant mean effect for total swimming speed (C10: 0.31 ± 0.01 m/s; C50: 0.31 ± 0.02 m/s; Con 0.29 ± 0.01 m/s; $F_{(2,20)}=0.08$; $p<0.93$).

3.1.2. The probe trial — memory test (Day 5, trial 17)

The number of crossings over the area of the previous platform position in the SE quadrant was different in tested groups (C10: 3.63 ± 0.82, C50: 2.5 ± 0.63 and Con: 0.72 ± 0.36) ($F_{(2,20)}=4.89$; $p<0.02$). A significant improvement was observed in the C10 group ($p<0.01$, Newman–Keuls test) compared with control group (Fig. 3a). The animals treated with *C. longa* extract spent more time in the target quadrant (SE), where the platform was situated during acquisition trials (C10: 24.82 ± 3.42 s, C50: 22.88 ± 2.72 s) than control animals (Con: 16.57 ± 1.46 s) ($F_{(2,20)}=2.36$, $p<0.12$). Post-hoc tests showed a significant increase in the C10 group ($p<0.05$, *t*-test) (Fig. 3b).

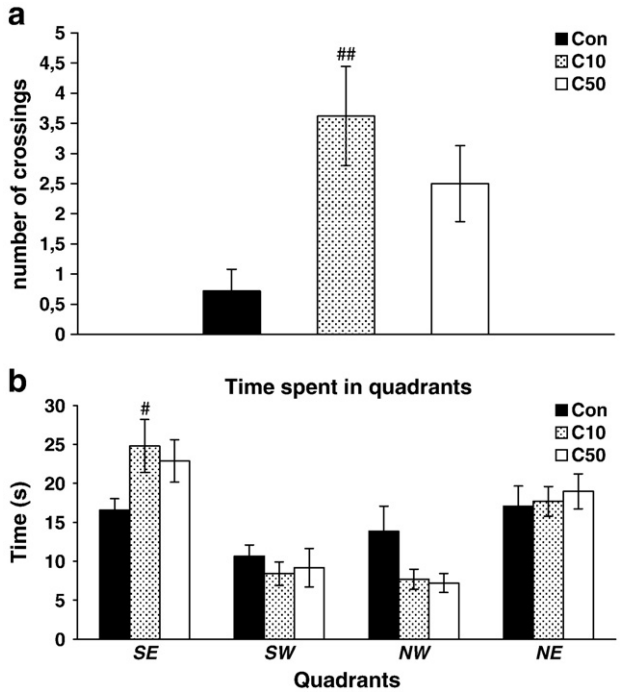

Fig. 3. Effects of 60 day pre-treatment with a standardised extract of *Curcuma longa* on learning and the memory of aged male Wistar rats in the water maze. a. The number of crossings (±SE) through the target area in a probe trial on Day 5 (trial 17) in the water maze for control and pre-treated rats, ##C10 vs control, $p<0.01$, NK. b. Time spent in quadrants (±SE) in a probe trial on Day 5 (trial 17) in the water maze for control and pre-treated rats, #C10 vs control, $p<0.05$, *t*-test. $F_{(2,20)}=2.36$, $p<0.12$.

3.1.3. Visible platform test (Day 6, trials 18–21)

An analysis of variance demonstrated significant differences between groups pre-treated with *C. longa* extract in comparison with the controls in escape latency in the cued task on Day 6 ($F_{(2,20)}=5.15$; $p<0.02$). In post-hoc analysis (Newman–Keuls test) the values of mean escape latency were significantly decreased by the extract at doses 10 mg/kg b.w. (14.64 ± 3.08 s; $p<0.03$) and 50 mg/kg b.w. (15.25 ± 2.40 s; $p<0.02$) compared to the control group (33.82 ± 4.38 s). The mean distance travelled was shortened in C10 (3.22 ± 0.58 m, $p<0.01$) and in C50 (4.00 ± 0.60 m; $p<0.02$) vs Con (8.15 ± 1.13 m) ($F_{(2,20)}=6.14$; $p<0.01$). The results did not show a significant main effect for swimming speed ($F_{(2,20)}=0.03$; $p<0.97$).

3.2. Biochemistry

3.2.1. Monoamine levels in selected brain regions

The levels of monoamines and their metabolites in the prefrontal cortex, hippocampus, hypothalamus and striatum are demonstrated in Table 1.

3.2.1.1. 5-HT (5-hydroxytryptamine). Statistically significant differences in 5-hydroxytryptamine content between the groups of rats were observed in the prefrontal cortex ($F_{(2,20)}=37.0$, $p<0.0001$ ANOVA), hippocampus ($F_{(2,20)}=11.18$, $p<0.0006$) and hypothalamus ($F_{(2,20)}=6.25$, $p<0.007$). Further analysis (Newman–Keuls test) proved a severe increase of 5-HT content in the C50 group in all the mentioned brain regions (prefrontal cortex: 546.99 ± 18.42 ng/g of wet tissue; $p<0.0001$, hippocampus: 430.60 ± 16.45 ng/g; $p<0.01$, hypothalamus: 817.8 ± 36.77 ng/g, $p<0.01$) compared with control animals (363.38 ± 15.65 ng/g, 330.01 ± 28.35 ng/g, 668.31 ± 28.59 ng/g respectively).

3.2.1.2. 5-HIAA (5-hydroxyindoloacetic acid). ANOVA also showed significant differences in hydroxyindoloacetic acid content in the prefrontal cortex ($F_{(2,20)}=7.96$, $p<0.003$), hippocampus ($F_{(2,20)}=7.44$, $p<0.004$) and hypothalamus ($F_{(2,20)}=4.65$, $p<0.02$). Rats treated with *C. longa* extract at the dose 50 mg/kg b.w. had an elevated content of this 5-HT metabolite in the prefrontal cortex (305.8 ± 20.3 ng/g of wet tissue, $p<0.01$), hippocampus (483.9 ± 33.1 ng/g, $p<0.01$) and hypothalamus (645.8 ± 48.3 ng/g, $p<0.05$) compared to standard fed animals (Con: 220.3 ± 10.6 ng/g, 355.4 ± 18.7 ng/g, 480.1 ± 19.8 ng/g respectively) (Newman–Keuls test).

3.2.1.3. 5HIAA/5-HT ratio. There were no differences in the ANOVA analysis in 5-hydroxytryptamine turnover (5-HIAA/5-HT) in any brain region ($F_{(2,20)}=2.1$; $p<0.15$).

3.2.1.4. DA (dopamine) and DOPAC (3, 4-dihydroxyphenylacetic acid). ANOVA showed differences between groups in dopamine ($F_{(2,20)}=4.51$, $p<0.02$) and its metabolite DOPAC ($F_{(2,20)}=5.38$, $p<0.013$) levels in the prefrontal cortex. The significantly reduced content of dopamine was found in the C50 group (DA: 35.7 ± 4.4 ng/g, $p<0.05$ Newman–Keuls test) in comparison to C10 (53.4 ± 4.3 ng/g) but not to control (48.5 ± 4.5 ng/g). The concentration of DOPAC in the C50 group (7.7 ± 0.7 ng/g, $p<0.05$ Newman–Keuls test) was lower than in control rats (Con: 15.0 ± 2.5 ng/g).

3.2.1.5. DOPAC/DA ratio. There were no differences in the ANOVA analysis in dopamine turnover (DOPAC/DA) in any brain region ($F_{(2,20)}=2.19$; $p<0.14$).

3.2.2. Amino acid levels in selected brain regions

The levels of amino acids in the prefrontal cortex, hippocampus, hypothalamus and striatum are given in Table 2.

3.2.2.1. Glutamic acid. In extract-treated animals the levels of glutamic acid in hippocampus were significantly decreased in comparison with

Table 1
Effects of chronic oral administration of a standardised extract of *Curcuma longa* on monoamine and metabolite levels (±SE) in brain regions in aged male rats. *C50 vs control, $p<0.05$, **C50 vs control, $p<0.01$, ****C50 vs control, $p<0.0001$, $^{\$}$C50 vs C10, $p<0.05$, $^{\$\$}$C50 vs C10, $p<0.01$, $^{\$\$\$}$C50 vs C10, $p<0.001$ (NK).

Monoamine and metabolite levels in ng/g of wet tissue	Brain region				
	Group	Prefrontal cortex	Hippocampus	Hypothalamus	Striatum
NA	Con	403.7 ± 11.7	470.1 ± 18.2	2197.9 ± 142.9	302.2 ± 44.7
	C10	391.1 ± 19.3	463.2 ± 24.4	1862.4 ± 213.5	302.2 ± 18.5
	C50	420.2 ± 28.4	499.1 ± 39.7	2132.2 ± 106.2	344.9 ± 32.6
HVA	Con	–	13.0 ± 5.2	–	231.6 ± 35.8
	C10	–	10.7 ± 3.1	–	193.5 ± 29.7
	C50	–	32.6 ± 16.1	–	217.9 ± 34.2
DA	Con	48.5 ± 4.5	275.9 ± 119.1	708.0 ± 75.3	10,250.5 ± 1918.6
	C10	53.4 ± 4.3	142.1 ± 44.5	996.6 ± 416.8	6730.2 ± 1350.8
	C50	35.7 ± 4.4$^{\$}$	606.6 ± 252.4	560.2 ± 34.3	7233.6 ± 802.0
DOPAC	Con	15.0 ± 2.5	29.1 ± 8.3	71.8 ± 9.3	794.0 ± 124.1
	C10	14.9 ± 2.0	17.6 ± 4.8	106.5 ± 46.2	603.1 ± 112.0
	C50	7.7 ± 0.7$^{*\$}$	69.5 ± 36.5	62.4 ± 6.3	626.8 ± 69.0
DOPAC/DA	Con	0.307 ± 0.043	0.131 ± 0.0259	0.101 ± 0.007	0.08 ± 0.003
	C10	0.277 ± 0.026	0.137 ± 0.0167	0.104 ± 0.005	0.096 ± 0.008
	C50	0.222 ± 0.015*	0.107 ± 0.015	0.110 ± 0.008	0.088 ± 0.007
5-HT	Con	363.4 ± 15.7	330.0 ± 28.3	668.3 ± 28.6	390.3 ± 45.1
	C10	401.9 ± 13.2	310.5 ± 13.3	660.3 ± 39.6	352.99 ± 37.2
	C50	546.9 ± 18.4$^{****\$\$\$}$	430.6 ± 16.4$^{**\$\$\$}$	817.8 ± 36.8$^{**\$\$}$	381.29 ± 18.0
5-HIAA	Con	220.3 ± 10.6	355.4 ± 18.7	480.1 ± 19.8	388.7 ± 30.5
	C10	252.8 ± 11.6	381.9 ± 18.5	544.2 ± 37.8	393.2 ± 27.1
	C50	305.8 ± 20.3**	483.9 ± 33.1$^{**\$\$}$	645.8 ± 48.3*	413.3 ± 29.2
5-HIAA/5-HT	Con	0.607 ± 0.018	1.105 ± 0.069	0.721 ± 0.021	1.028 ± 0.079
	C10	0.632 ± 0.032	1.229 ± 0.023	0.823 ± 0.032	1.156 ± 0.076
	C50	0.557 ± 0.029	1.117 ± 0.05	0.789 ± 0.045	1.081 ± 0.056

naturally aged rats (C10: 9.12 ± 0.18 μmol/g of wet tissue; $p<0.05$, Newman–Keuls test, C50: 9.318 ± 0.186 μmol/g; $p<0.05$, Newman–Keuls test vs Con: 10.153 ± 0.447 μmol/g) ($F_{(2,20)}=3.62$, $p<0.05$).

3.2.2.2. Taurine. The levels of taurine were different among all the groups tested both in the hippocampus ($F_{(2,20)}=4.38$, $p<0.03$) and in the striatum ($F_{(2,20)}=7.37$, $p<0.004$). The significant decrease of taurine concentration was observed in the hippocampus in the group C10 (3.955 ± 0.122 μmol/g; $p<0.05$, Newman–Keuls test) but not in C50 (4.202 ± 0.153 μmol/g; NS $p>0.05$) vs control animals (4.626 ± 0.204 μmol/g). In the striatum a lower concentration of taurine was seen in C10 (4.699 ± 0.147 μmol/g; $p<0.01$, Newman–Keuls test) and C50 (5.086 ± 0.147 μmol/g; $p<0.05$ Newman–Keuls test) than compared with Con (5.606 ± 0.123 μmol/g).

3.2.3. Plasma corticosterone levels

Levels of plasma corticosterone were significantly decreased in rats treated with the extract (C10: 70.75 ± 13.4 ng/ml; $p=0.05$ Newman–

Table 2
Effects of chronic oral administration of a standardised extract of *Curcuma longa* on the levels of amino acids (±SE) in brain regions in aged male rats. $^{\#}$C10 vs control, $p<0.05$, $^{\#\#}$C10 vs control, $p<0.01$, *C50 vs control, $p<0.05$, $^{\$}$C50 vs C10, $p<0.05$ (NK).

Amino acids levels in μmol/g of wet tissue	Brain region				
	Group	Prefrontal cortex	Hippocampus	Hypothalamus	Striatum
Glutamine	Con	4.831 ± 0.235	5.407 ± 0.331	5.457 ± 0.0865	4.984 ± 0.378
	C10	4.455 ± 0.223	4.779 ± 0.236	5.233 ± 0.176	4.875 ± 0.196
	C50	5.154 ± 0.159	5.253 ± 0.132	5.649 ± 0.178	5.539 ± 0.125
Glutamic acid	Con	10.697 ± 0.246	10.153 ± 0.447	7.343 ± 0.273	8.598 ± 0.699
	C10	10.395 ± 0.126	9.12 ± 0.18$^{\#}$	7.76 ± 0.256	9.628 ± 0.283
	C50	10.725 ± 0.203	9.318 ± 0.186*	7.64 ± 0.190	9.672 ± 0.235
Taurine	Con	4.57 ± 0.212	4.626 ± 0.204	2.34 ± 0.0887	5.51 ± 0.157
	C10	4.017 ± 0.157	3.955 ± 0.122$^{\#}$	2.395 ± 0.282	4.729 ± 0.174$^{\#\#}$
	C50	4.262 ± 0.105	4.202 ± 0.153	2.152 ± 0.0682	4.959 ± 0.097*
Alanine	Con	0.655 ± 0.0127	0.621 ± 0.0581	0.375 ± 0.0125	0.592 ± 0.072
	C10	0.609 ± 0.0209	0.582 ± 0.0229	0.396 ± 0.0185	0.515 ± 0.256
	C50	0.696 ± 0.0224$^{\$}$	0.647 ± 0.0315	0.391 ± 0.0216	0.552 ± 0.022
GABA	Con	2.123 ± 0.0585	2.467 ± 0.0972	5.198 ± 0.231	2.05 ± 0.284
	C10	2.012 ± 0326	2.442 ± 0.113	4.953 ± 0.136	1.787 ± 0.071
	C50	2.152 ± 0.0494	2.626 ± 0.164	5.191 ± 0.314	1.954 ± 0.083
Aspartic acid	Con	3.181 ± 0.1	2.085 ± 0.0994	2.25 ± 0.101	1.818 ± 0.17
	C10	2.947 ± 0.977	1.87 ± 0.075	2.22 ± 0.047	2.062 ± 0.107
	C50	3.095 ± 0.0977	1.892 ± 0.0476	2.385 ± 0.106	2.117 ± 0.077
Glycine	Con	0.683 ± 0.0315	0.88 ± 0.0339	1.170 ± 0.0155	0.793 ± 0.118
	C10	0.676 ± 0.0329	0.911 ± 0.0676	1.147 ± 0.0662	0.675 ± 0.027
	C50	0.653 ± 0.0296	0.813 ± 0.0221	1.093 ± 0.055	0.646 ± 0.022
Histidine	Con	0.0755 ± 0.00595	0.0782 ± 0.0059	0.079 ± 0.000449	0.401 ± 0.327
	C10	0.0755 ± 0.00595	0.0794 ± 0.00455	0.0843 ± 0.00352	0.073 ± 0.004
	C50	0.0723 ± 0.00309	0.073 ± 0.00182	0.0736 ± 0.00343	0.067 ± 0.002

Table 3
Effects of chronic oral administration of standardised extract of *Curcuma longa* (CPE-014) on the mean plasma corticosterone concentrations (±SE) in aged male rats. #C10 vs control, $p<0.05$, **C50 vs control, $p<0.01$ (NK).

Group	N	Mean plasma corticosterone concentrations (ng/ml)
Con	7	105.714 ± 10.014
C10	8	70.75 ± 13.421#
C50	8	52.5 ± 11.488**

Keuls test, C50: 52.5 ± 11.49 ng/ml; $p=0.01$ Newman–Keuls test) in comparison with age-matched controls (105.71 ± 10.01 ng/ml) ($F_{(2,20)}=4.98$; $p<0.018$) (Table 3).

3.2.4. Neurotransmitter levels and memory correlation

The number of crossings over the previous position of the platform in the first probe test was compared with the levels of amino acids, monoamines and their metabolites in the selected regions of the rats' brains.

There was no correlation between amino acids levels and the number of crossings in the first probe test in the prefrontal cortex and hippocampus. It has been shown that glycine ($r=-0.731$, $p=0.04$, $F_{(1,6)}=6.9$) and glutamine ($r=-0.755$, $p=0.03$, $F_{(1,6)}=7.97$) concentrations of the C50 group in the striatum negatively correlated with the results of the probe test.

There was no correlation between the number of crossings and monoamines, their metabolites levels in the first probe trial in the hippocampus. Prefrontal cortex 5-HT levels correlated positively with the crossings of the C10 ($r=0.831$, $p=0.01$, $F_{(1,6)}=13.36$) and C50 ($r=0.695$, $p=0.05$, $F_{(1,6)}=5.6$) groups during the probe trial (Fig. 4). The concentrations of dopamine ($r=-0.799$, $p=0.02$, $F_{(1,6)}=10.63$) and DOPAC ($r=-0.848$, $p=0.008$, $F_{(1,6)}=15.41$) in the striatum of the C10 group correlated negatively with the number of crossings in the probe trial.

4. Discussion

The data of this study have confirmed that extract of *C. longa* is able to improve memory in aged rats. Curcumin is a major active component of the tested standardised rhizome extract of this plant. The results of our

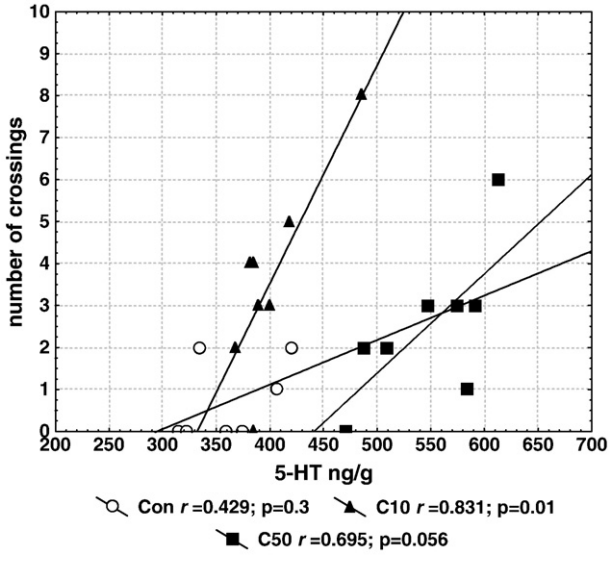

Fig. 4. Correlations between the number of crossings over the position of a hidden platform in the Memory Test on the 5th day of the experiment and the concentration of serotonin (5-HT) in the cortex in control rats and pre-treated with a standardised extract of *Curcuma longa*. r — Pearson's correlation coefficients.

investigations are consistent with the reports of curcumin-induced memory improvement in animal models of AD (Frautschy et al., 2001), depression (Wang et al., 2008), chronic stress (Xu et al., 2009), in rats with haloperidol-induced orofacial dyskinesia (Bishnoi et al., 2008), brain ischemia (Al-Omar et al., 2006), brain injury (Wu et al., 2006) and diabetic encephalopathy (Kuhad and Chopra, 2007). Conboy et al. (2009) noticed recently, that synthetic curcumin (diferuloylmethane), given into the stomach by a gavage, at the dose of 75 mg for 8 days, improves learning and memory in 18 month old Wistar rats in the water maze.

In our experiment long-term oral administration of the extract resulted in enhanced ability of spatial learning as well as memory in aged rats in the water maze.

It is broadly known that curcumin exerts multiple neuroprotective effects but detailed mechanisms should be still established.

Normal aging results in alterations in brain neurotransmission systems — the reduction of monoamine concentrations, enzyme activity involved in their synthesis and diminished receptor sensitivity. Monoamine turnover may be increased to prevent depletion in their levels. In an old rat brain a decrease in the number of serotoninergic neurons and changed morphology were described (Nishimura et al., 1995). Schmitt et al. (2006), in experimental trials has observed that cognitive performance may be dysregulated by the modulation of serotonin transmission. Reduced serotonin turnover resulted in diminished memory functions and cognitive ability. Curcumin is able to influence the MAO activity in the brain (Yu et al., 2002; Kulkarni et al., 2008; Bhutani et al., 2009) and consequently change the concentration of neurotransmitters: noradrenaline (NA), dopamine (DA), serotonin (5-HT) and their metabolites in central nervous system. Curcumin can reverse the decrease of 5-HT, 5-HIAA, NA, and DA in a rat brain due to olfactory bulbectomy (Xu et al., 2005) and stress conditions (the mouse forced swimming test) (Xia et al., 2007; Kulkarni et al., 2008; Bhutani et al., 2009).

The serotonin system seems to be essential for emotional stress and memory processes. The hippocampus and amygdala control emotional behaviour and are connected with anxiety. 5-Hydroxytryptamine plays an important role in the pathology of depression and serotoninergic receptors are pivotal in antidepressant responses. In the forced swimming test in mice the serotoninergic system seems to be involved in the antidepressant-like effect of curcumin, which is possibly mediated thanks to interaction with 5HT1A/1B and 5HT-2C receptors (Xu et al., 2007; Wang et al., 2008) and improvement of adenylyl cyclase-cAMP pathway (Li et al., 2009).

In this study we have discovered that *C. longa* extract significantly modified 5-hydroxytryptamine concentration in the prefrontal cortex and hippocampus — structures that are related to spatial and stimulus–response learning. Curcumin-treated animals had shorter latencies both to the hidden and the visible platform in the water maze. Hence it is concluded that not only the hippocampus but also other regions of the brain (e.g. prefrontal cortex) are responsible for better learning and memory functions. In view of the better results of pre-treated animals in the cued version of the water maze it is possible to assume that curcumin may also influence some sensorimotor functions. In this research extract pre-treatment had no influence on speed of swimming in the water maze. This suggests that curcumin may improve motivation of tested animals in behavioural performance. The improved learning ability, better spatial and cued navigation may be the results of enhanced serotonin neurotransmission. Increased serotonin levels in the prefrontal cortex correlated positively with crossings over the target space during the first probe trial. Such brain regions as prefrontal cortex, hippocampus and striatum are innervated by 5-HT neurons from the raphe complex and are involved in cognitive processes. Research in experimental animals strongly suggests that stimulation of 5HT receptors in prefrontal cortex can ameliorate spatial working memory related cognitive deficits, and may even enhance cognitive function in healthy

animals (González-Burgos and Feria-Velasco, 2008). These findings may be of a direct relevance to our study.

Stress conditions result in elevated plasma corticosterone. It is also known that long-term exposure to stress or glucocorticoid excess impairs memory function (Sapolsky et al., 1986; McEwen, 1999; Roozendaal, 2002). The aged animals pre-treated with *C. longa* extract that underwent behavioural examination had significantly lowered the plasma corticosterone levels compared to the control swimming rats in our experiment. This is consistent with an observation that stress-induced increases in serum corticosterone concentrations were reduced in curcumin-treated rats (Xu et al., 2006; Li et al., 2009; Xu et al., 2009). Long-term administration of curcumin may reverse the changes caused by the chronic stress in rats' adrenal glands. Xu et al. (2006) noticed that abnormal gland weight to body weight ratio, increased thickness of the adrenal cortex as well as reduced glucocorticoid receptor mRNA expression were reversed by chronic oral curcumin administration. It is also known that curcumin decreases the proinflammatory brain interleukin-1-beta levels (Lim et al., 2001) and in this way may affect the release of CRF (corticotropin releasing factor) in the hypothalamus. Comparably the NSAIDs (e.g. ibuprofen) lower the corticosterone levels by the impact on IL-6 and ACTH concentrations (Turnbull and Rivier, 1996; Aydin et al., 2002).

Aging is connected with growing tissue oxidative stress, which may be a reason for impaired cellular calcium homeostasis. It is broadly known that a rise in the glutamate-dependent intracellular calcium influx is a last stage of the common pathway of a few cell-injurious stimuli and causes the activation of pro-oxidant and protease systems that lead in the end to neuronal death. Glutamic acid is an excitatory amino acid, and excessive neuronal secretion yields excitotoxicity involved in the pathogenesis of neurodegeneration. Curcumin blocks calcium intracellular entry (Matteucci et al., 2005) and protects against glutamate cytotoxicity in studies in vitro (Suh et al., 2007). The lowered glutamate release, and consequently the decrease in calcium intracellular concentration may explain the neuroprotective effect of chronic curcumin administration. A depletion in serotonin can be correlated with increased glutamate transmission. Curcumin dose-dependently prevents the decreased level of serotonin and hence may limit the excitotoxicity and oxidative damage induced by the depletion of serotonin in the brain (Bishnoi et al., 2008; Bhutani et al., 2009). In our research hippocampal glutamate levels were significantly reduced in both groups of curcumin-fed aged rats. Hence curcumin diminishes hippocampal glutamate concentration and consequently neuronal overexcitation with increased cell calcium influx then it may exert neuroprotective activity.

Glutamic acid enhances the release of taurine in different brain regions (Del Arco et al., 2001). Taurine may modulate the excitatory effects of glutamate, and protect against glutamate-induced cytotoxicity thanks to influence on intracellular calcium homeostasis (Foos and Wu, 2002). Taurine acts also as an antioxidant (Oja and Saaransari, 2007), hence it may exert neuroprotective effects. Stress conditions or kindling enhance brain taurine levels (Ebner et al., 2005; Szyndler et al., 2008). Exogenous taurine presents anxiolytic-like effects in behavioural trials in rodents (Chen et al., 2004; Kong et al., 2006). It is presumed that this may be mediated by the interaction of taurine with serotonin and the inhibitory GABA system. Taurine is a $GABA_A$ receptor agonist (El Idrissi and Trenkner, 2004). The exogenous taurine administration improves learning and retention in aged mice (El Idrissi, 2008) but does not affect the memory function in rats (Ito et al., 2009). The lowered brain taurine concentrations in our study need further examination. The administration of *C. longa* extract to aged rats in our study led to diminished concentrations of taurine in the hippocampus and striatum, which are brain regions described as especially rich in this amino acid (Galarreta et al., 1996). The significant depletion of taurine was observed in the hippocampus of rats that were given the smaller dose of curcumin (10 mg/kg) but the greater dose (50 mg/kg) caused a statistically insignificant reduction. Both pre-treated groups had diminished striatal taurine concentrations compared to controls. The decreased hippocampal level of taurine may result from diminished glutamate activity in this brain structure. It is possible that controls may have elevated brain taurine level, connected with greater stress response reflected by enhanced plasma corticosterone and diminished brain serotonin concentration, however still lower than younger rats as compared with available literature data. Dawson et al. (1999) noticed a significant decrease in taurine levels in hippocampus, cortex, striatum and hypothalamus in healthy 18 month old rats in comparison to 12 month old ones. The present data indicate that curcumin-induced decrease in taurine concentration is not directly related to the behavioural effects of the curcuma extract, and may be considered an epiphenomenon.

In our research on aged animals the differences between groups in dopamine concentrations in the prefrontal cortex were observed (ANOVA). However none of the experimental group when analysed in post-hoc tests had the significantly different dopamine level compared to control. In C10 group vs Con the concentration of dopamine was not changed significantly. Reduced content of dopamine in the C50 group vs C10 and DOPAC in the C50 group vs Con was seen, however there were no differences in dopamine turnover (DOPAC/DA) in any brain region. The absence of any significant alterations in dopamine metabolism in the striatum suggests that curcumin does not directly affect the motor functions of pre-treated animals as it was also seen in behavioural performance. The influence of curcumin administration on dopamine level was already tested in younger rodents. The elevated dopamine concentrations in whole brain tissue after administration of 40–80 mg of curcumin i.p. were observed under stress conditions (Kulkarni et al., 2008; Bhutani et al., 2009). Xu et al. (2005) indicated that in olfactory bulbectomy depression model in young animals the level of dopamine in prefrontal cortex was elevated after administration of 10 mg/kg of curcumin however the influence of the greater dose was not analysed.

It is plausible that in aged animals the ability of curcumin pre-treatment to affect dopamine levels is on the whole diminished.

The results of our study indicate that the various effects of *C. longa* extract administration depend on the dose. Some effects may be greater after administration of the greater dose (e.g. the decrease of plasma corticosterone levels or the increase of 5-HT concentration in the prefrontal cortex) but others are weaker and could be described as an example of hormesis as non-linear, biphasic activity of the extract was observed (Calabrese and Baldwin, 2001). This may contain the influence on brain glutamic acid and taurine levels. The hippocampal glutamate and striatal taurine levels were affected by both doses of the extract, however the greater dose had weaker effect there, and in hippocampus had no significant effect on taurine concentration. Some preclinical studies support the biphasic action of curcumin on cells survival suggesting the induction of the mechanisms of cell death connected with broad pleiotropic activity of curcumin on multiple intracellular targets (Salvioli et al., 2007).

In conclusion our study revealed that curcumin may cause the behavioural and neurochemical changes in aged animals due to its influence of the serotoninergic and glutaminergic systems, and that curcumin pre-treatment may lead to enhanced tolerance to stress conditions.

Acknowledgement

The authors are indebted to Labofarm, Poland for providing the *Curcuma longa* extract.

References

Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol 2009;41:40–59.

Al-Omar FA, Nagi MN, Abdulgadir MM, Al Joni KS, Al-Majed AA. Immediate and delayed treatments with curcumin prevents forerain ischemia-induced neuronal damage and oxidative insult in the rat hippocampus. Neurochem Res 2006;31:611–8.

Aydin O, Aldemir M, Taçyildiz I, Girgin S, Sen T, Baç B. The metabolic and hormonal effects of perioperative ibuprofen treatment on surgical stress. Ulus Travma Derg 2002;8:6-10.
Bala K, Tripathy BC, Sharma D. Neuroprotective and anti-ageing effects of curcumin in aged rat brain regions. Biogerontology 2006;7:81–9.
Bhutani MK, Bishnoi M, Kulkarni SK. Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. Pharmacol Biochem Behav 2009;92:39–43.
Bishnoi M, Chopra K, Kulkarni SK. Protective effect of Curcumin, the active principle of turmeric (*Curcuma longa*) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in rat brain. Pharmacol Biochem Behav 2008;88:511–22.
Calabrese EJ, Baldwin LA. Hormesis: U-shaped dose responses and their centrality in toxicology. Trends Pharmacol Sci 2001;221:285–91.
Chen SW, Kong WX, Zhang YJ, Li YL, Mi XJ, Mu XS. Possible anxiolytic effects of taurine in the mouse elevated plus-maze. Life Sci 2004;75:1503–11.
Conboy L, Foley AG, O´Boyle NM, Lawlor M, Gallagher HC, Murphy KJ, et al. Curcumin-induced degradation of PKCδ is associated with enhanced dentate NCAM PSA expression and spatial learning in adult and aged Wistar rats. Biochem Pharmacol 2009;77:1254–65.
Daniel S, Limson JL, Dairam A, Watkins GM, Daya S. Through metal binding, curcumin protects against lead- and cadmium-induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain. J Inorg Biochem 2004;98:266–75.
Dawson Jr R, Liu S, Eppler B, Patterson T. Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats. Mech Ageing Dev 1999;107:73–91.
Del Arco A, Segovia G, Prieto L, Mora F. Endogenous glutamate–taurine interaction in striatum and nucleus accumbens of the freely moving rat: studies during the normal process of aging. Mech Ageing Dev 2001;122:401–14.
Ebner K, Bosch OJ, Krömer SA, Singewald N, Neumann ID. Release of oxytocin in the rat central amygdala modulates stress-coping behavior and the release of excitatory amino acids. Neuropsychopharmacology 2005;30:223–30.
El Idrissi A, Trenkner E. Taurine as a regulator of excitatory and inhibitory neurotransmission. Neurochem Res 2004;29:189–97.
El Idrissi A. Taurine improves learning and retention in aged mice. Neurosci Lett 2008;436:19–22.
Foos TM, Wu JY. The role of taurine in the central nervous system and the modulation of intracellular calcium homeostasis. Neurochem Res 2002;27:21–6.
Frautschy SA, Hu W, Kim P, Miller SA, Chu T, Harris-White ME, et al. Phenolic anti-inflammatory antioxidant reversal of Abeta-induced cognitive deficits and neuropathology. Neurobiol Aging 2001;22:993-1005.
Galarreta M, Bustamante J, Martin del Rio R, Solis JM. Taurine induces a long-lasting increase of synapsis efficacy and axon excitatibility in hippocampus. J Neurosci 1996;16:92-102.
Garcia-Alloza M, Borrelli LA, Rozkalne A, Hyman BT, Bacskai BJ. Curcumin labels amyloid pathology in vivo, disrupts existing plaques and partially restores distorted neurites in an Alzheimer mouse model. J Neurochem 2007;102:1095–104.
Ghoneim AI, Abdel-Naim AB, Khalifa AE, El-Denshary ES. Protective effects of curcumin against ischemia/reperfusion insult in rat forebrain. Pharmacol Res 2002;46:273–9.
Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain: the disposition of H(3)-norepinephrine, H(3)-dopamine and H(3)-DOPA in various regions of the brain. J Neurochem 1966;13:655–69.
Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. Biochem Pharmacol 2008;75:787–809.
González-Burgos I, Feria-Velasco A. Serotonin/dopamine interaction in memory formation. Review Prog Brain Res 2008;172:603–23.
Hiserodt R, Hartman TG, Ho CT, Rosen RT. Characterization of powdered turmeric by liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry. J Chromatogr A 1996;740:51–63.
Hong J, Bose M, Ju J, Ryu JH, Chen XC, Sang S, et al. Modulation of arachidonic acid metabolism by curcumin and related b-diketone derivatives: effects on cytosolic phospholipase A2, cyclooxygenases and 5-lipooxygenase. Carcinogenesis 2004;25: 1671–9.
Ito K, Arko M, Kawaguchi T, Kuwahara M, Tsubone H. The effect of subacute supplementation of taurine on spatial learning and memory. Exp Anim 2009;58:175–80.
Jacob A, Wu R, Zhou M, Wang P. Mechanism of the anti-inflammatory effect of curcumin: PPAR-γ activation. PPAR Res 2007. doi:10.1155/2007/89369.
Jiang J, Wang W, Sun YJ, Hu M, Li F, Zhu DY. Neuroprotective effect of curcumin on focal cerebral ischemic rats by preventing blood–brain barrier damage. Eur J Pharmacol 2007;561:54–62.
Kaneda N, Asano M, Nagatsu T. Simple method for the simultaneous determination of acetylcholine, choline, noradrenaline, dopamine and serotonin in brain tissue by high-performance liquid chromatography with electrochemical detection. J Chromatogr 1986;360:211–8.
Khopde SM, Priyadarsini KI, Venkatesan P, Rao MN. Free radical scavenging ability and antioxidant efficiency of curcumin and its substituted analogue. Biophys Chem 1999;80:85–91.
Kong WX, Chen SW, Li YL, Zhang YJ, Wang R, Min L, et al. Effects of taurine on rat behaviors in three anxiety models. Pharmacol Biochem Behav 2006;83:271–6.
Kuhad A, Chopra K. Curcumin attenuates diabetic encephalopathy in rats: behavioral and biochemical evidences. Eur J Pharmacol 2007;576:34–42.
Kulkarni SK, Bhutani MK, Bishnoi M. Antidepressant activity of curcumin: involvement of serotonin and dopamine system. Psychopharmacology 2008;201:435–42.
Li YC, Wang FM, Pan Y, Qiang LQ, Cheng G, Zhang WY, et al. Antidepressant-like effects of curcumin on serotonergic receptor-coupled AC-cAMP pathway in chronic unpredictable mild stress of rats. Prog Neuropsychopharmacol Biol Psychiatry 2009;33:435–49.
Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 2001;21:8370–7.
Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life Sci 2006;78:2081–7.
Matteucci A, Frank C, Domenici MR, Balduzzi M, Paradisi S, Carnovale-Scalzo G, et al. Curcumin treatment protects rat retinal neurons against excitotoxicity: effect on N-methyl-D-aspartate-induced intracellular Ca^{2+} increase. Exp Brain Res 2005;167:641–8.
McEwen B. Stress and the aging hippocampus. Front Neuroendocrinol 1999;20:49–70.
Mercier S, Frederic C, Buguet A, Cespuglio R, Martin S, Bourdon L. Behavioural changes after an acute stress: stressor and test types influences. Behav Brain Res 2003;139: 167–75.
Nishimura A, Ueda S, Takeuchi Y, Sawada T, Kawata M. Age-related decrease of serotonergic fibres and S-100 beta immunoreactivity in the rat dentate gyrus. NeuroReport 1995;6:1445–8.
Oja SS, Saaransari P. Pharmacology of taurine. Proc West Pharmacol Soc 2007;50:8-15.
Quiles JL, Mesa MD, Ramirez-Tortosa CL, Aguilera CM, Battino M, Gil A, et al. *Curcuma longa* extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. Arterioscler Thromb Vasc Biol 2002;22:1225–31.
Ramirez-Tortosa MC, Mesa MD, Aguilera MC, Quiles JL, Baro L, Ramirez-Tortosa CL, et al. Oral administration of turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. Atherosclerosis 1999;147:371–8.
Roozendaal B. Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiol Learn Mem 2002;78:578–95.
Rowley HL, Martin KF, Marsden CA. Determination of in vivo amino acid neurotransmitters by high-performance liquid chromatography with o-phthalaldehyde-sulphite derivatisation. J Neurosci Methods 1995;57:93–9.
Salvioli S, Sikora E, Cooper EL, Franceschi C. Curcumin in cell death processes: a challenge for CAM of age-related pathologies. Evid Based Complement Alternat Med 2007;4:181–90.
Sandur SK, Ichikawa H, Pandey MK, Kunnumakkara AB, Sung B, Sethi G, et al. Role of pro-oxidants and antioxidants in the anti-inflammatory and apoptotic effects ofcurcumin (diferuloylmethane). Free Radic Biol Med 2007;43:568–80.
Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr Rev 1986;7:284–301.
Schmitt JA, Wingen M, Ramaekers JG, Evers EA, Riedel WJ. Serotonin and human cognitive performance. Curr Pharm Des 2006;12:2473–86.
Suh HW, Kang S, Kwon KS. Curcumin attenuates glutamate-induced HT22 cell death by supressing MAP kinase signaling. Moll Cell Biochem 2007;298:187–94.
Szyndler J, Maciejak P, Turzynska D, Sobolewska A, Lehner M, Taracha E, et al. Changes in the concentration of amino acids in the hippocampus of pentylenetetrazole-kindled rats. Neurosci Lett 2008;439:245–9.
Turnbull AV, Rivier C. Corticotropin-releasing factor, vasopressin, and prostaglandins mediate, and nitric oxide restrains, the hypothalamic–pituitary–adrenal response to acute local inflammation in the rat. Endocrinology 1996;137:455–63.
Wang R, Xu Y, Wu HL, Li YB, Li YH, Guo JB, et al. The antidepressant effect of curcumin in the forced swimming test involve 5-HT_1 and 5-HT_{-2} receptors. Eur J Pharmacol 2008;578:43–50.
Widy-Tyszkiewicz E, Scheel-Kruger J, Christensen V. Spatial navigation learning in spontaneously hypertensive, renal hypertensive and normotensive Wistar rats. Behav Brain Res 1993;54:179–85.
Wu A, Ying Z, Gomez-Pinilla F. Dietary curcumin counteracts the outcome of traumatic brain injury on oxidative stress, synaptic plasticity and cognition. Exp Neurol 2006;197:309–17.
Xia X, Cheng G, Pan Y, Xia ZH, Kong LD. Behavioral, neurochemical and neuroendocrine effects of ethanolic extract from *Curcuma longa* L. in the mouse forced swimming test. J Ethnopharmacol 2007;110:356–63.
Xu Y, Ku BS, Yao HY, Lin YH, Ma X, Zhang YH, et al. Antidepressant effects of curcumin in the forced swim test and olfactory bulbectomy models of depression in rats. Pharmacol Biochem Behav 2005;82:200–6.
Xu Y, Ku BS, Tie L, Yao H, Jiang W, Ma X, et al. Curcumin reverses the effect of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. Brain Res 2006;1122:56–64.
Xu Y, Ku B, Cui L, Li X, Barish PA, Foster TC, et al. Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. Brain Res 2007;1162:9-18.
Xu Y, Lin D, Li S, Li G, Shyamala SG, Barish PA, et al. Curcumin reverses impaired cognition and neuronal plasticity induced by chronic stress. Neuropharmacology 2009;57:463–71.
Yamamoto H, Hanada K, Kawasaki K, Nishijima M. Inhibitory effect of curcumin on mammalian phospholipase D activity. FEBS Lett 1997;417:196–8.
Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, et al. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques and reduces amyloid in vivo. J Biol Chem 2005;280:5892–901.
Yu ZF, Kong LD, Chen Y. Antidepressant activity of aequous extracts of *Curcuma longa* in mice. J Ethnopharmacol 2002;83:161–5.
Zafir A, Banu N. Antioxidant potential of fluoksetine in comparison to *Curcuma longa* in restaint-stressed rats. Eur J Pharmacol 2007;572:23–31.
Zhao J, Zhao Y, Zheng W, Lu Y, Feng G, Yu S. Neuroprotective effect of curcumin on transient focal cerebral ischemia in rats. Brain Res 2008;1229:224–32.