Tualang honey supplement improves memory performance and hippocampal morphology in stressed ovariectomized rats

Badriya Al-Rahbi [a], Rahimah Zakaria [a,*], Zahiruddin Othman [b], Asma Hassan [c], Zul Izhar Mohd Ismail [c], Sangu Muthuraju [d]

[a] Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Malaysia
[b] Department of Psychiatry, School of Medical Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Malaysia
[c] Department of Anatomy, School of Medical Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Malaysia
[d] Department of Neuroscience, School of Medical Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Malaysia

ARTICLE INFO

ABSTRACT

Recently, our research team has reported that Tualang honey was able to improve immediate memory in postmenopausal women comparable with that of estrogen progestin therapy. Therefore the aim of the present study was to examine the effects of Tualang honey supplement on hippocampal morphology and memory performance in ovariectomized (OVX) rats exposed to social instability stress. Female Sprague-Dawley rats were divided into six groups: (i) sham-operated controls, (ii) stressed sham-operated controls, (iii) OVX rats, (iv) stressed OVX rats, (v) stressed OVX rats treated with 17β-estradiol (E2), and (vi) stressed OVX rats treated with Tualang honey. These rats were subjected to social instability stress procedure followed by novel object recognition (NOR) test. Right brain hemispheres were subjected to Nissl staining. The number and arrangement of pyramidal neurons in regions of CA1, CA2, CA3 and the dentate gyrus (DG) were recorded. Two-way ANOVA analyses showed significant interactions between stress and OVX in both STM and LTM test as well as number of Nissl-positive cells in all hippocampal regions. Both E2 and Tualang honey treatments improved both short-term and long-term memory and enhanced the neuronal proliferation of hippocampal CA2, CA3 and DG regions compared to that of untreated stressed OVX rats.

Introduction

Functional integrity of brain regions including the hippocampus proper, dentate gyrus (DG), amygdala, entorhinal, perirhinal, and parahippocamal cortices is necessary for object recognition memory processing (Clarke, 2000; Brown and Aggleton, 2001). Hippocampal CAl, CA3 and DG comprise the hippocampal formation (Eichenbaum et al., 1994) which has long been regarded as a crucial structure for memory (Olton et al., 1989; Hodges, 1995).

Chronic stress and its associated prolonged chronic elevation of glucocorticoid hormones have negative effects on cognitive abilities of animals such as learning and memory by causing neuronal death and reducing neurogenesis (Sapolsky, 1992; McEwen and Sapolsky, 1995; McEwen, 2000). It has been suggested that prolonged chronic elevation of glucocorticoid hormones should result in structural differences in the hippocampus such as reduced volume, fewer hippocampal neurons and reduced neurogenesis (McEwen and Sapolsky, 1995; Gould and Tanapat, 1999; Ohl and Fuchs, 1999).

On the other hand, estrogens have a beneficial influence on the morphological and electrophysiological properties of the hippocampus (Leuner et al., 2004), a brain region implicated in certain forms of learning, memory and stress. Estrogen increased astrocytic volume in the rat CA1 (Klintsova et al., 1995) which in turn provides trophic support for the formation of new dendritic spines and synapses, and increased Nissl-positive cells in hippocampal CA3 and DG regions (Takuma et al., 2007). It has been shown in experimental studies using rats that exposure to estrogen, either exogenously or endogenously, during proestrus enhances the density of dendritic spines in several areas of the hippocampus (Gould et al., 1990; Zhang et al., 1999). Estrogen treatment also protects against a wide range of toxic insults including free radical generators (Behl et al., 2000), excitotoxicity (Singer et al., 1999; Singh et al., 1999; Diaz Brinton et al., 2000), β-amyloid-induced toxicity (Green and Simpkins, 2000) and ischemia (Zhang et al., 1998; Viscoli et al.,

2001). Both estrogen receptors, ERα and ERβ, are expressed in the hippocampus and cortex of rodent and human brain (Beyer, 1999; Green and Simpkins, 2000; Shughrue and Merchenthaler, 2000; Milner et al., 2001, 2005; Lu et al., 2003) and their activation (mainly ERα) can promote neuron survival in rat hippocampal neurons (Zhao et al., 2004).

Nevertheless, the evidence for a neuroprotective role of hormone replacement therapy (HRT) for cognitive disorders in menopausal women has been inconclusive. Previous meta-analyses of research and epidemiological studies suggested that HRT might have a beneficial effect on cognition in postmenopausal women (Hogervorst et al., 2000; Marder and Sano, 2000). However, subsequent reports of the Women's Health Initiative Memory Study (WHIMS) in very large cohorts (Espeland et al., 2004; Shumaker et al., 2004; Resnick et al., 2006) stated that HRT increased the risk of cognitive impairment and dementia in elderly women. However, recent studies on postmenopausal women reported beneficial effects of HRT on verbal memory (Tierney et al., 2009; Othman et al., 2011).

Despite reports on the beneficial effects of estrogen treatment (MacLennan et al., 2001; Dören et al., 2003; Sherwin and Henry, 2008; Rozenberg et al., 2013), many postmenopausal women refuse to take HRT to alleviate their menopausal symptoms. A substantial number of women have discontinued its use because of side-effects such as withdrawal bleeding, bloating, premenstrual irritability, lower abdominal cramps, and breast tenderness or increased risk of cancer or other HRT-linked conditions (Ettinger et al., 1999, 2003; Regan et al., 2001; Hulley et al., 2002; Lacey et al., 2002; Miller et al., 2002; Beral et al., 2003; Shumaker et al., 2003). Numerous alternatives to HRT are being researched to relieve postmenopausal symptoms and improve quality of life (Borrelli and Ernst, 2010; Cardini et al., 2010; Lunny and Fraser, 2010; Othman et al., 2011; Asltoghiri and Ghodsi, 2012).

Malaysian Tualang honey is a pure wild multifloral honey produced by Asian rock bees, *Apis dorsata* (Khalil et al., 2011). The bees build immense honeycombs on branches of very tall Tualang trees (*Koompassia excelsa*) in the Rain Forest of Northern Peninsular Malaysia (Bashkaran et al., 2011). Honey contains significant antioxidant activities (Frankel et al., 1998; Al-Mamary et al., 2002; Fahey and Stephenson, 2002; Gheldof et al., 2002; Aljadi and Kamaruddin, 2004; Beretta et al., 2005; Inoue et al., 2005; Blasa et al., 2006; Nagai et al., 2006; Beretta et al., 2007; Perez et al., 2007; Brudzynski and Miotto, 2011; Kishore et al., 2011) as well as choline and acetylcholine (Heitkamp, 1984) which are essential for brain function and as neurotransmitters. A previous animal study reported that honey-fed rats performed significantly better than those fed sucrose or a sugar-free diet in the Y maze task (Chepulis et al., 2009). Recently, our research team has reported that Tualang honey (*Agro Mas*) was able to improve immediate memory in postmenopausal women comparable with that of estrogen progestin therapy (Othman et al., 2011).

Therefore the aim of the present study was to examine the effects of Tualang honey (*Agro Mas*) supplement on hippocampal morphology and memory performance in overiectomized rats exposed to social instability stress.

Materials and methods

Animals

Sixty adult female Sprague-Dawley rats of approximately 8-weeks old, with body weight of 200 ± 20 g, were obtained from the Laboratory Animal Research Unit, Universiti Sains Malaysia (USM). All rats were housed in polypropylene cages (40 cm × 25 cm × 16 cm), exposed to 12 h light–dark cycles, maintained at a room temperature of 23 °C, and provided with free access to food and water. The experimental protocol was approved by the Research and Ethics Committee, USM.

The rats were randomly divided into six groups ($n = 10$ per group). These were: (1) sham-operated control rats, (2) stressed sham-operated control rats, (3) ovariectomized (OVX) rats, (4) stressed OVX rats, (5) stressed OVX rats treated with 17β-estradiol (20 μg daily, subcutaneously) and (6) stressed OVX rats treated orally with Tualang honey (0.2 g/kg body weight).

Surgical procedures

Forty rats underwent bilateral OVX through a dorsal incision under anesthesia (90 mg/kg ketamine and 5 mg/kg xylazine, intraperitoneally). The other 20 rats were sham-operated, i.e. the ovaries were not removed. After the operation, the rats were kept in individual cages to avoid any interactions which might lead to bleeding or poor wound healing for 10 days. The groups were reformed and all the rats were left undisturbed for two months as a recovery period.

Social instability stress procedure

The social instability stress procedure was conducted eight weeks after ovariectomy. The stress procedure consists of alternating isolation and crowding phases for 15 days as previously described (Haller et al., 1999). The experiment started and ended with an isolation phase, and each phase lasted for 24 h. Eight rats (three males and five females) were held per cage for a crowding phase. Behavior of rats was videotaped for the initial 30 min of each crowding phase. Biting attacks, dominant postures and fighting for food were counted (De Goeij et al., 1992). Body weights were recorded at the end of each crowding phase.

Animal treatments

The rats were treated with either 17β-estradiol (Cayman Chemical, Ann Arbor, MI, USA) 20 μg/day in 2.5 μl corn oil injected subcutaneously (Takuma et al., 2007) or Tualang honey (Agro Mas, Federal Agricultural Marketing Authority (FAMA), Mergong, Kedah, Malaysia) 0.2 g/kg body weight/day administered orally by gavage diluted in 1 ml of distilled water (Zaid et al., 2010) three days prior to stress procedure and the treatments were continued throughout the 15 days of stress procedure.

Behavioral test

The novel object recognition (NOR) test uses the natural preference for novel object displayed by rats. This test is normally used to assess cognitive alterations associated with aging, genetic manipulations, or drug treatments. The chamber was an open field apparatus (60 cm × 60 cm × 30 cm). Firstly, all animals were submitted to a habituation session for three days during which they were placed in the empty open field and left to freely explore the field for 10 min. During the training session, two identical objects (A1 and A2) were placed in the field, and the rat was allowed to explore freely for 10 min as described in previous studies (De Lima et al., 2005; Pieta Dias et al., 2007). Time spent exploring each object was recorded manually. For test sessions, animals were tested for memory retention 2 h after training session (short-term memory/retention, STM). In STM test, the rats explored the open field for 5 min in the presence of one familiar (A1 or A2) and one novel (B) object. The location of objects was alternated with each new animal; it was approximately placed in 50% trials in the right side and 50% in the left side of the field. The same test was repeated

24 h after the training session and this is known as long-term memory/retention, LTM.

All objects consisted of plastic toys and had a height of about 5 cm. Objects presented similar textures, colors and sizes, but distinctive shapes. The objects were positioned in two adjacent corners, 10 cm from the walls. Between tests, the objects were cleaned with 10% ethanol solution to mask any olfactory cues.

Exploration was defined as sniffing or touching the object with the nose. Sitting on the object was not considered as exploration (Bowman et al., 2002). Total exploration times of the familiar and novel objects were recorded and used to calculate a discrimination index [time spent with novel object (B) − time spent with familiar object (A)]/[total time exploring both objects] for training and test sessions (Reneerkens et al., 2012). This index was used to measure recognition memory (Kamei et al., 2006). The exploration of each object was expressed as percentage of total exploration time. Increased exploration time of the novel object or better preference to novel object was interpreted as successful retention of memory for the familiar object. An absence of any difference in the exploration of the two objects was interpreted as memory deficit (Carlini et al., 2008).

Blood and tissue collection

The animals were sacrificed by decapitation immediately after the NOR sessions. Blood samples (10 ml) were collected immediately. All blood samples were left to clot for 2 h prior to centrifugation for 15 min at 4000 rpm (EBA 21, Hettich GmbH & Co. KG, Tuttlingen, Germany). Approximately 3 ml of serum was collected and stored at −20 °C until assay. The brain of each animal was quickly removed followed by careful dissection of the right brain hemispheres in ice-cold saline. The hemispheres were then stored in 10% formalin until assayed.

Estimation of serum corticosterone levels

Serum corticosterone levels were measured using a specific ELISA kit (Creative Diagnostics, Shirley, NY, USA) according to the manufacturer's instructions. Briefly, 100 μl of serum sample was added into each well followed by 100 μl of enzyme-labeled corticosterone. The plate was incubated at 37 °C for 90 min. Following incubation, the wells were carefully washed. 100 μl of biotin-antibody working solution was added into each well and then incubated at 37 °C for 60 min. After three washes, 100 μl of horseradish peroxidase (HRP) was added into each well and then incubated at 37 °C for 30 min. Next, 100 μl of 3,3′,5,5′-tetramethylbenzidine (TMB) reagent was added into each well and then incubated at room temperature for 20 min, which resulted in the development of color change. The color development was then stopped with the addition of 100 μl of stop solution. The absorbance was measured at 450 nm using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Nissl staining

The right brain hemispheres were embedded in paraffin wax, cut into 5-μm-thick coronal sections using a rotary microtome (HM505E; Microm International GmbH, Walldorf, Germany), mounted on slides and followed by Nissl staining. Nissl staining was performed according to the standard procedure (Yamada et al., 2005). Two continuous fields of each hippocampus region were selected and captured (Olympus CX41 biological microscope; Tokyo, Japan). The arrangement of pyramidal neurons in regions of CA1, CA2, CA3 and the DG was recorded. The Nissl-positive cells were counted at different magnifications using High Definition Medical Image Analysis Program (analySIS docu 5.0, Münster,

Germany). The mean of two fields was taken as the number of Nissl-positive cells for each section and the mean of four sections was taken as the Nissl-positive cells of each group. Cells which had a shrunken or unclear body with surrounding empty spaces were excluded.

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). Probability values less than 5% ($P < 0.05$) were considered statistically significant. Two-way analyses of variance (ANOVA) were utilized to examine the main effects of social stress (stressed vs. unstressed) and surgery (sham-operated vs. OVX) on NOR behavior and the number of Nissl-positive cells in hippocampal regions.

Data on NOR behavior, the number of Nissl-positive cells in hippocampal regions and corticosterone levels were analyzed using a one-way ANOVA and where appropriate, Tukey's post hoc tests were utilized to determine group differences. Pearson's Correlation Coefficient was utilized to test the correlation between the number of Nissl-positive cells in hippocampal regions and discrimination indexes during STM and LTM tests.

Results

Effects of surgery and social stress on NOR behavior and the number of Nissl-positive cells in hippocampal regions

A two-way ANOVA was conducted to determine whether if exposed to social stress and surgery (OVX) affects the NOR behaviors and number of Nissl-positive cells in hippocampal regions. A significant main effect of surgery (OVX) was observed for the discriminative index during STM ($F(1,36) = 11.21$, $P < 0.05$) and LTM ($F(1,36) = 61.50$, $P < 0.001$) tests, with OVX rats had lower discrimination index during both STM and LTM tests compared to those non-OVX rats. A significant main effect of surgery (OVX) was also observed for the number of Nissl positive cells in hippocampal CA1 ($F(1,36) = 5.44$, $P < 0.05$), CA2 ($F(1,36) = 39.25$, $P < 0.001$), CA3 ($F(1,36) = 49.11$, $P < 0.001$) and DG ($F(1,36) = 19.81$, $P < 0.001$) regions, indicating that OVX rats had lower number of Nissl-positive cells in all the hippocampal regions compared to those non-OVX rats.

A significant main effect of stress was observed for the discriminative index during LTM test ($F(1,36) = 33.68$, $P < 0.001$) but not during STM test ($F(1,36) = 3.75$, $P = 0.061$), indicating that stressed rats had lower discrimination index during LTM test compared to those unstressed rats. A significant main effect of stress was also observed for the number of Nissl positive cells in CA1 ($F(1,36) = 10.77$, $P < 0.05$), CA3 ($F(1,36) = 8.12$, $P < 0.05$), but not in CA2 ($F(1,36) = 2.17$, $P = 0.149$) and DG ($F(1,36) = 2.13$, $P = 0.153$), indicating that stressed rats had a lower number of Nissl-positive cells in hippocampal CA1 and CA3 regions compared to those unstressed rats.

With regard to the interaction between surgery (OVX) and stress, the discrimination index during both STM and LTM tests, and the number of Nissl-positive cells in hippocampal CA1, CA2, CA3 and DG regions were significant ($F(1,36) = 14.33$, $P < 0.05$; $F(1,36) = 25.37$, $P < 0.001$; $F(1,36) = 12.51$, $P < 0.05$ and $F(1,36) = 12.81$, $P < 0.05$; respectively), indicating that both OVX and stress were associated with a decline in the short-term and long-term memory performance as well as reduction in the number of Nissl-positive cells in all the hipppocampal regions.

Effects of E2 and Tualang honey on memory performance

Further analyses to examine the effects of E2 and Tualang honey in stressed OVX rats on memory performance were conducted
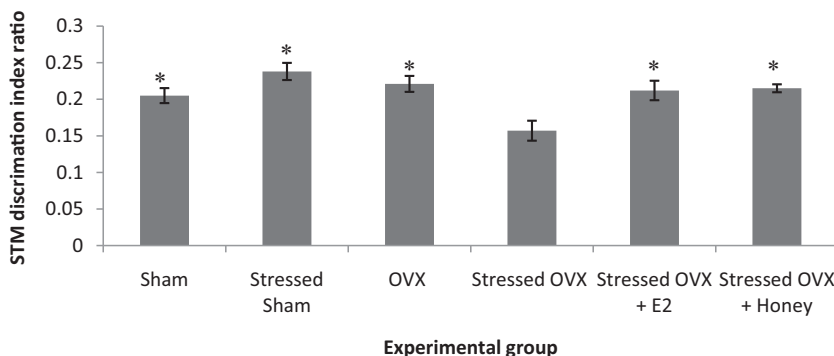
Fig. 1. Discrimination index during STM test ($n = 10$). Discrimination indices are expressed as mean ratio [time spent with new object novel − time spent with object familiar]/[total time exploring both objects] ± SEM. *$P < 0.05$ compared with the stressed OVX group.

using one-way ANOVA. The one way ANOVA showed discrimination indices during STM and LTM tests were significantly different between the groups ($F(5,54) = 5.95$, $P < 0.001$ and $F(5,54) = 40.66$, $P < 0.001$, respectively). The post hoc data indicated that stressed OVX treated with either E2 or Tualang honey have a significantly higher discrimination index during both STM (Fig. 1) and LTM (Fig. 2) tests compared to untreated stressed OVX rats. There was no difference in the discrimination indices during STM and LTM tests between stressed OVX treated with E2 compared to that of the Tualang honey group ($P > 0.05$). Both E2 and Tualang honey treated groups improved STM comparable to the sham-operated control group but not for LTM.

Effects of E2 and Tualang honey on the number of Nissl-positive cells in hippocampal regions

One-way ANOVA showed numbers of Nissl-positive cells in CA1, CA2, CA3 and DG hippocampal regions were significantly different between groups ($F(5,54) = 6.66$, $P < 0.001$; $F(5,54) = 19.84$, $P < 0.001$; $F(5,54) = 26.58$, $P < 0.001$; $F(5,54) = 6.54$, $P < 0.001$; respectively). The post hoc data indicated that stressed OVX treated with either E2 or Tualang honey have a considerably higher number of Nissl-positive cells in hippocampal CA2 (Fig. 3B), CA3 (Fig. 3C) and DG (Fig. 3D) regions compared to untreated stressed OVX rats. There was no comparable difference in the number of Nissl-positive cells in all the hippocampal regions between stressed OVX treated with E2 compared to that of the Tualang honey group.
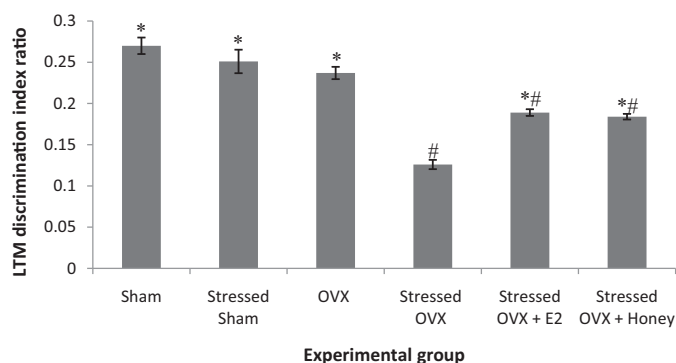

Fig. 2. Discrimination index during LTM test ($n = 10$). Discrimination indices are expressed as mean ratio [time spent with new object novel − time spent with object familiar]/[total time exploring both objects] ± SEM. *$P < 0.05$ compared with the stressed OVX group; #$P < 0.05$ compared with the sham group.

Nissl staining results

Nissl staining revealed that the arrangement of hippocampal CA2 (Fig. 4) and CA3 (Fig. 5) pyramidal neurons of stressed or unstressed sham-operated controls were trimmed and dense, and the Nissl substance in cytoplasm was clearly visible. The arrangement of pyramidal neurons of untreated stressed OVX group was sparse and the Nissl substance decreased or dissolved (Figs. 4D and 5D). The arrangement of hippocampal CA2 (Fig. 4E and F) and CA3 (Fig. 5E and F) pyramidal neurons of stressed treated OVX groups was more regular than that of the untreated stressed OVX group as shown in Figs. 4D and 5D.

Serum corticosterone levels

One way ANOVA results revealed that corticosterone levels differed notably amongst the groups ($F(5, 54) = 62.11$, $P < 0.001$) (Fig. 6). The corticosterone levels were highest in the stressed OVX group, followed by the unstressed OVX, stressed sham-operated control and unstressed sham-operated control groups. The post hoc analysis revealed a significant decrease in corticosterone levels in the stressed OVX rats following treatment with either E2 or Tualang honey comparable to that of stressed sham-operated controls. These findings indicated that E2 and Tualang honey could reduce the stress levels to a certain extent as shown by the corticosterone levels which were comparable to that of stressed sham-operated controls but the levels were still significantly high when compared to sham-operated controls.

Correlation between discrimination indices during STM and LTM tests, and the number of hippocampal neurons

There were significant positive correlations between discrimination indices during the STM test and the number of CA2 ($r = 0.35$, $P < 0.05$), CA3 ($r = 0.35$, $P < 0.05$) and DG ($r = 0.58$, $P < 0.001$). Stronger correlations were noted between discrimination indices during the LTM test and the number of CA2 ($r = 0.60$, $P < 0.001$), CA3 ($r = 0.76$, $P < 0.001$) and DG ($r = 0.57$, $P < 0.001$). However, there were no significant correlations between the number of CA1 and discrimination indices during STM tests ($r = -0.13$, $P > 0.05$) and LTM test ($r = -0.14$, $P > 0.05$).

Discussion

In the present study, we used overiectomized rat as a menopause model to examine the effects of estrogen deficiency on hippocampal morphology and memory performance. The memory performance was assessed using the NOR test because an earlier study showed that recognition memory (object recognition test)
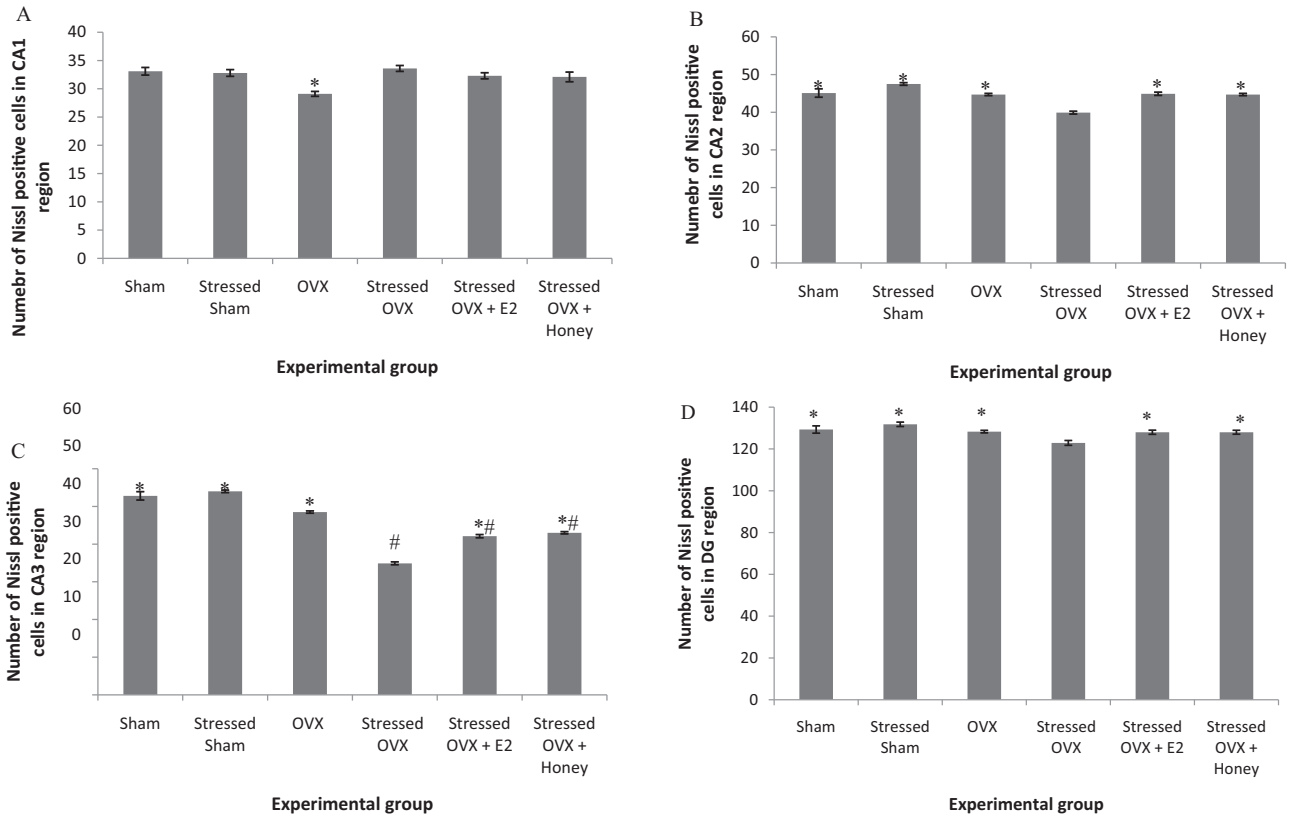
Fig. 3. Mean number of Nissl-positive cells in (A) CA1, (B) CA2, (C) CA3 and (D) DG hippocampal regions (mean ± SEM per 0.01 mm^2). *$P<0.05$ compared with the stressed OVX group; #$P<0.05$ compared with the sham group.

Fig. 4. The arrangement of hippocampal CA2 pyramidal neurons between the groups. (A) Sham-operated control, (B) stressed sham-operated control; (C) OVX; (D) stressed OVX, (E) stressed OVX treated with E2 and (F) stressed OVX treated with Tualang honey. The arrow indicates dead or damage cells (Nissl staining × 200; scale bar = 100 μm).
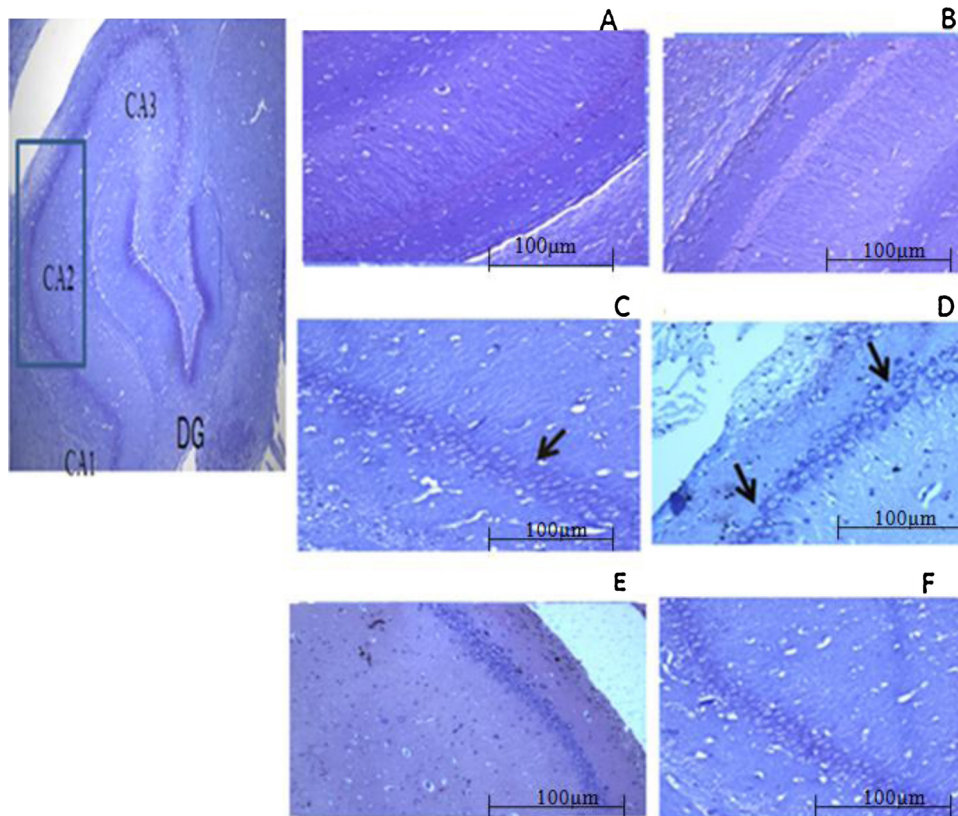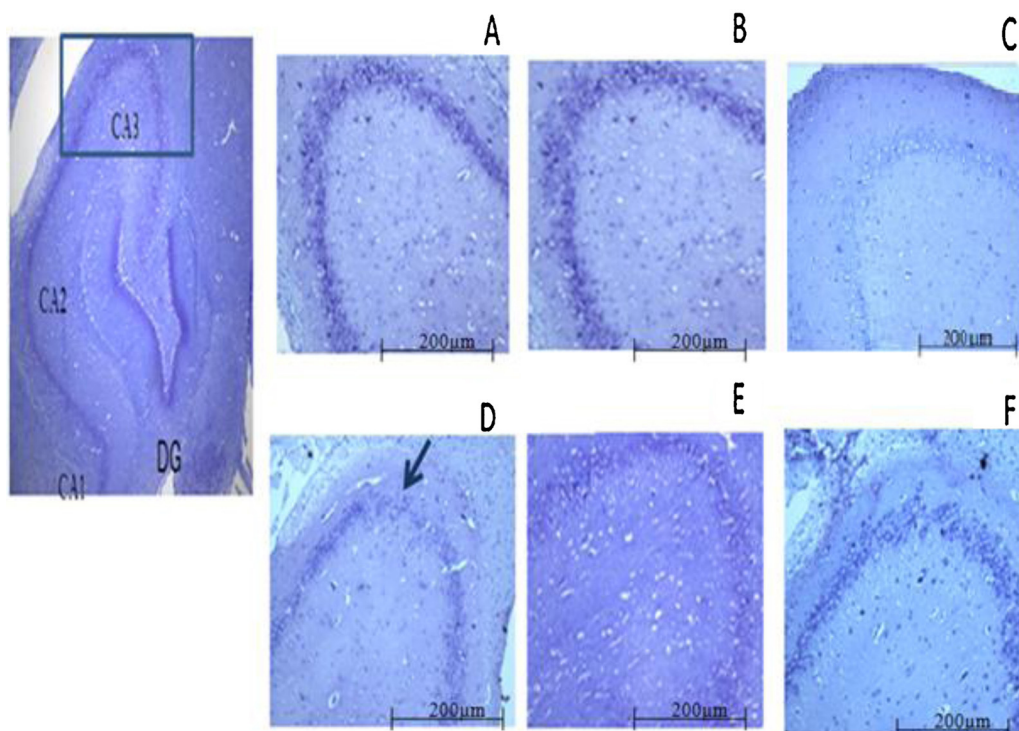
Fig. 5. The arrangement of hippocampal CA3 pyramidal neurons between the groups. (A) Sham-operated control, (B) stressed sham-operated control; (C) OVX; (D) stressed OVX, (E) stressed OVX treated with E2 and (F) stressed OVX treated with Tualang honey. The arrow indicates dead or damage cells (Nissl staining × 100; scale bar = 200 μm).

is more sensitive to ovarian steroids than spatial memory (object placement test) and performance of object recognition test was lost faster after OVX (Luine, 2008) as compared to object placement test.

Memory formation is a process that requires multiple steps, including acquisition, cellular consolidation, and system consolidation. Memories are first formed in a labile state, and then the memory trace is stabilized through the process of consolidation (McGaugh, 1966) via molecular and/or structural modifications (Kandel, 2001). Processing of a memory trace can be divided into at least two phases: short-term memory (0–3 h), a protein synthesis-independent phase that lasts minutes to hours, and long-term memory, a protein synthesis-dependent phase that lasts from hours to days to weeks (Izquierdo et al., 1998; Kandel, 2001; Medina et al., 2008). In this study, NOR test was conducted twice, the first test was 2 h after the training session which represents the STM and the second test was 24 h after training session which represents the LTM. In the STM test, the object recognition was based on the acquisition (learning) and this process occurs in the prefrontal

cortex (PFC) (Warden and Miller, 2010) and the hippocampus was not required at this stage. In contrast to the LTM test, the object recognition required retrieval from consolidated memory and this process occurred in the hippocampus.

Our two-way ANOVA analyses showed that the long-term memory performance and Nissl-positive cell numbers in hippocampal CA1 and CA3 regions were affected by stress. These findings were consistent with those of earlier reports which showed long term memory impairment (Conrad et al., 1996) and degenerating cells in the CA2 and CA3 sub-areas (Jain et al., 2001) in stressed rats. Our findings, however, revealed that stress did not affect short-term memory and Nissl-positive cell numbers in hippocampal CA2 and DG regions. This could be explained by the fact that the effects of stress on synaptic plasticity and memory performance depend on the stressor timing, intensity and duration (Cazakoff et al., 2010). An earlier study showed that when a high dose of corticosterone administered for 10 min before and during a high frequency tetanus, hippocampal synaptic potentiation was facilitated (Wiegert et al., 2006). Therefore it is possible to postulate at this stage that the effect of stress was not so severe compared to OVX to affect the entire hippocampal areas and the prefrontal cortex, which is important for short-term memory.

Our biochemical results confirmed that serum corticosterone levels were highest in OVX rats exposed to social instability stress for 15 days compared to those who were not exposed to stress. Two-way ANOVA revealed that the corticosterone levels were higher in OVX rats compared to stressed rats. The corticosterone levels were reduced following treatment with either E2 or Tualang honey in stressed OVX rats comparable to that of stressed sham-operated controls but were significantly higher than unstressed sham-operated controls. These suggest possible interaction between E2 and Tualang honey with the HPA axis.

In the present study, OVX exerts significant effects on both short-term and long-term memory performances, and arrangement and Nissl-positive cell numbers in hippocampal CA1, CA2, CA3 and DG regions. Our data clearly indicated that there was memory
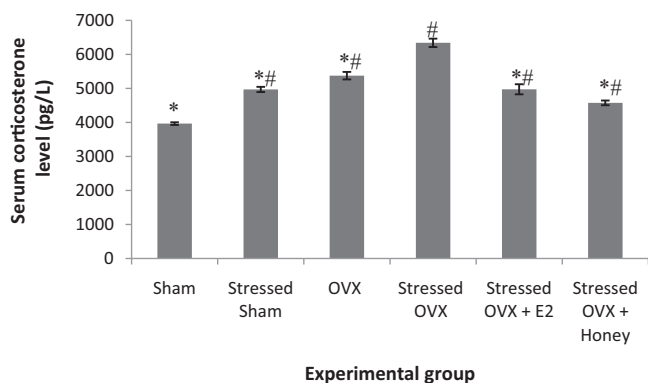

Fig. 6. Mean serum corticosterone level ($n = 8$). $^*P < 0.05$ compared with the stressed OVX group; $^\#P < 0.05$ compared with the sham group.

deficit both short-term and long-term as well as significant reduction in the number of Nissl-positive cells in all hippocampal regions in OVX rats when compared to sham-operated controls. Our findings revealed that estrogen deficiency and higher stress levels as shown by higher corticosterone in OVX rats compared to sham-operated controls, caused negative effects, not only on the whole hippocampal regions, but possibly PFC, thus, caused both short-term and long-term memory deficit. These findings were further supported by significant positive correlations between discrimination indices of STM and LTM tests, and the Nissl-positive cell numbers in hippocampal CA2, CA3 and DG regions. Our findings were supported by recent findings in rats that showed memory impairment beginning in the second month after ovariectomy and the histological data revealed high damage to CA3 area (Su et al., 2012). Our results remain inconsistent, however, with those of previous studies (Gould et al., 1990; Woolley and Schwartzkroin, 1998) who found a decreased dendritic spine density in CA1, but not in CA3 following OVX. It could be explained by the difference in the duration of post-OVX and the estradiol levels. In that study, the rats were killed after one week post-OVX as compared to nearly 3 months post-OVX in our study. However, the estradiol levels were not available for comparison (Gould et al., 1990).

Our findings disclosed substantial interactions (stress × OVX) in both STM and LTM test as well as number of Nissl-positive cells in all hippocampal regions, indicating that both social stress and OVX influences the memory performance as well as hippocampal neurons. Therefore, the stressed OVX model was used to examine the effect of E2 and Tualang honey treatments on the NOR behavior and hippocampal morphology. Our data showed that both E2 and Tualang honey treatments were able to improve both short-term and long-term memory performances as indicated by higher discrimination indices compared to those of stressed OVX rats without treatment. The histological data confirmed that both E2 and Tualang honey treatments in stressed OVX rats were able to enhance the neuronal proliferation in hippocampal CA2, CA3 and DG regions. Taken together, these findings suggest that Tualang honey treatment was able to exert the same beneficial effects on memory performance and hippocampal morphology comparable to that of E2 treatment.

Our findings were supported by previous studies which showed E2 treatment improved the cognitive in object recognition task (Luine et al., 2003; Li et al., 2004; Walf et al., 2006; Scharfman et al., 2007) and morphological impairments in the hippocampus (Takuma et al., 2007) of rats and mice. Previous studies have suggested that E2 mediated its neuroprotective effects through its antioxidant properties (Mukai et al., 1990; Komuro et al., 1990; Vedder et al., 1999), up-regulation of brain-derived neurotrophic factor (BDNF) expression (Takuma et al., 2007) which may lead to activation of ERs-mediated cell survival signaling pathways (Yu et al., 2004; Carrer et al., 2005) and augmentation of choline acetyltransferase and acetylcholinesterase activities in specific brain areas (Luine, 1985; Gibbs, 1996; Gibbs et al., 2002). Tualang honey may share similar mechanisms of neuroprotection with E2 as it has high antioxidant properties, i.e. total phenolic content was 251.7 ± 7.9 mg gallic acid/kg honey, total antioxidant activity was 322.1 ± 9.7 (μM Fe(II)), the antiradical activity was 41.30 ± 0.78 (% inhibition) (Mohamed et al., 2010). However, further studies are needed to confirm the choline and acetylcholine activities in Tualang honey and the neuroprotective mechanisms of Tualang honey.

There are a few limitations with the present study. Firstly, as previously noted, apart from the hippocampus, the prefrontal cortex is also a target for estrogen action (Markham and Greenough, 2004; Tang et al., 2004; Wallace et al., 2006). In our study, we only examined the hippocampal morphology, but not the PFC and thus could not exclude the effect of E2 and Tualang honey on the PFC.

Secondly, we used Nissl staining to study neuronal morphology of the hippocampus. The Nissl staining approach allows the visualization of all somata, but not neuronal processes in appropriately prepared tissue sections. A more powerful method may be utilized in the future using a combined approach of Golgi silver impregnation technique and the Nissl staining method. This method will allow the establishment of the detailed morphological profiles of neurons within a nucleus or a laminar structure (Pilati et al., 2008).

In conclusion, our study demonstrates that stressed OVX rats negatively affect STM and LTM as well as the hippocampal morphology. E2 and Tualang honey improved the memory and hippocampal morphological impairments possibly through their antioxidant properties, by up regulation of BDNF expression or augmentation of choline acetyltransferase and acetylcholinesterase activities in specific brain areas or a combination of these mechanisms. The neuroprotective actions of Tualang honey support its potential used as an alternative therapy to help prevent memory decline in post-menopausal women.

References

Aljadi AM, Kamaruddin MY. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chem 2004;85:513–8.

Al-Mamary M, Al-Meeri A, Al-Habori M. Antioxidant activities and total phenolics of different types of honey. Nutr Res 2002;22:1041–7.

Asltoghiri M, Ghodsi Z. The effects of reflexology on sleep disorder in menopausal women. Procedia: Soc Behav Sci 2012;31:242–6.

Bashkaran K, Zunaina E, Bakiah S, Sulaiman SA, Sirajudeen K, Naik V. Anti-inflammatory and antioxidant effects of Tualang honey in alkali injury on the eyes of rabbits: experimental animal study. BMC Complement Altern Med 2011;11:90.

Behl C, Moosmann B, Manthey D, Heck S. The female sex hormone oestrogen as neuroprotectant: activities at various levels. Neuronal Cognit Effects Oestrogens 2000;230:221–38.

Beral V, Million Women Study Collaborators. Breast cancer and hormone replacement therapy in the Million Women Study. Lancet 2003;362:419–27.

Beretta G, Orioli M, Facino RM. Antioxidant and radical scavenging activity of honey in endothelial cell cultures (EA. hy926). Planta Med: Natural Prod Med Plant Res 2007;73:1182–9.

Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal Chim Acta 2005;533:185–91.

Beyer C. Estrogen and the developing mammalian brain. Anat Embryol 1999;199:379–90.

Blasa M, Candiracci M, Accorsi A, Piacentini M, Albertini M, Piatti E. Raw *Millefiori* honey is packed full of antioxidants. Food Chem 2006;97:217–22.

Borrelli F, Ernst E. Alternative and complementary therapies for the menopause. Maturitas 2010;66:333–43.

Bowman R, Ferguson D, Luine V. Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. Neuroscience 2002;113:401–10.

Brown MW, Aggleton JP. Recognition memory: what are the roles of the perirhinal cortex and hippocampus? Nat Rev Neurosci 2001;2:61–2.

Brudzynski K, Miotto D. The recognition of high molecular weight melanoidins as the main components responsible for radical-scavenging capacity of unheated and heat-treated Canadian honeys. Food Chem 2011;125:570–5.

Cardini F, Lesi G, Lombardo F, van der Sluijs C, MSCG – Menopause Survey Collaborative Group. The use of Complementary and Alternative Medicine by women experiencing menopausal symptoms in Bologna. BMC Women's Health 2010;10:7.

Carlini V, Martini A, Schiöth H, Ruiz R, Fiol de Cuneo M, De Barioglio S. Decreased memory for novel object recognition in chronically food-restricted mice is reversed by acute ghrelin administration. Neuroscience 2008;153:929–34.

Carrer HF, Cambiasso MJ, Gorosito S. Effects of estrogen on neuronal growth and differentiation. J Steroid Biochem Mol Biol 2005;93:319–23.

Cazakoff BN, Johnson KJ, Howland JG. Converging effects of acute stress on spatial and recognition memory in rodents: a review of recent behavioural and pharmacological findings. Prog Neuro-Psychopharm Biol Psychiatry 2010;34:733–41.

Chepulis LM, Starkey NJ, Waas JR, Molan PC. The effects of long-term honey, sucrose or sugar-free diets on memory and anxiety in rats. Physiol Behav 2009;97:359–68.

Clarke RP. Does longer-term memory storage never become overloaded, and would such overload cause Alzheimer's disease and other dementia? Med Hypoth 2000;55:419–28.

Conrad CD, Galea LAM, Kuroda Y, McEwen BS. Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine treatment. Behav Neurosci 1996;110:1321–34.

De Goeij DC, Dijkstra H, Tilders FJ. Chronic psychosocial stress enhances vasopressin, but not corticotropin-releasing factor, in the external zone of the median eminence of male rats: Relationship to subordinate status. Endocrinology 1992;131:847–53.

De Lima MNM, Laranja DC, Bromberg E, Roesler R, Schröder N. Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. Behav Brain Res 2005;156:139–43.

Diaz Brinton R, Chen S, Montoya M, Hsieh D, Minaya J, Kim J, et al. The women's health initiative estrogen replacement therapy is neurotrophic and neuroprotective. Neurobiol Aging 2000;21:475–96.

Dören M, Nilsson JA, Johnell O. Effects of specific post-menopausal hormone therapies on bone mineral density in post-menopausal women: a meta-analysis. Hum Reprod 2003;18:1737–46.

Eichenbaum H, Otto T, Cohen NJ. Two functional components of the hippocampal memory system. Behav Brain Sci 1994;17:449–72.

Espeland MA, Rapp SR, Shumaker SA, Brunner R, Manson JAE, Sherwin BB, et al. Conjugated equine estrogens and global cognitive function in postmenopausal women. JAMA 2004;291:2959–68.

Ettinger B, Grady D, Tosteson ANA, Pressman A, Macer JL. Effect of the Women's Health Initiative on women's decisions to discontinue postmenopausal hormone therapy. Obstet Gynecol 2003;102:1225–32.

Ettinger B, Pressman A, Silver P. Effect of age on reasons for initiation and discontinuation of hormone replacement therapy. Menopause 1999;6:282–9.

Fahey JW, Stephenson KK. Pinostrobin from honey and Thai ginger (*Boesenbergia pandurata*): a potent flavonoid inducer of mammalian phase 2 chemoprotective and antioxidant enzymes. J Agric Food Chem 2002;50:7472–6.

Frankel S, Robinson GE, Berenbaum MR. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. J Apicultural Res 1998;37:27–31.

Gheldof N, Wang XH, Engeseth NJ. Identification and quantification of antioxidant components of honeys from various floral sources. J Agric Food Chem 2002;50:5870–7.

Gibbs RB. Fluctuations in relative levels of choline acetyltransferase mRNA in different regions of the rat basal forebrain across the estrous cycle: effects of estrogen and progesterone. J Neurosci 1996;16:1049–55.

Gibbs RB, Nelson D, Anthony MS, Clarkson TB. Effects of long-term hormone replacement and of tibolone on choline acetyltransferase and acetylcholinesterase activities in the brains of ovariectomized, cynomologous monkeys. Neuroscience 2002;113:907–14.

Gould E, Tanapat P. Stress and hippocampal neurogenesis. Biol Psychiatry 1999;46:1472–9.

Gould E, Woolley CS, Frankfurt M, McEwen BS. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. J Neurosci 1990;10:1286–91.

Green PS, Simpkins JW. Neuroprotective effects of estrogens: potential mechanisms of action. Int J Dev Neurosci 2000;18:347–58.

Haller J, Fuchs E, Halász J, Makara GB. Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. Brain Res Bull 1999;50:33–9.

Heitkamp K. Pro und kontra Honig – Sind Aussagen zur Wirkung des Honigs "wissenschaftlich hinreichend gesichert"? Schriften Zur Oecotrophol 1984:1–60.

Hodges J. Memory, amnesia and the hippocampal system. J Neurol Neurosurg Psychiatry 1995;58:128.

Hogervorst E, Williams J, Budge M, Riedel W, Jolles J. The nature of the effect of female gonadal hormone replacement therapy on cognitive function in post-menopausal women: a meta-analysis. Neuroscience 2000;101:485–512.

Hulley S, Furberg C, Barrett-Connor E, Cauley J, Grady D, Haskell W, et al. Noncardiovascular disease outcomes during 6.8 years of hormone therapy: heart and estrogen/progestin replacement study follow-up (HERS II). JAMA 2002;288:58–66.

Inoue K, Murayarna S, Seshimo F, Takeba K, Yoshimura Y, Nakazawa H. Identification of phenolic compound in manuka honey as specific superoxide anion radical scavenger using electron spin resonance (ESR) and liquid chromatography with coulometric array detection. J Sci Food Agric 2005;85:872–8.

Izquierdo I, Barros DM, Souza TM, De Souza MM, Izquierdo LA, Medina JH. Mechanisms for memory types differ. Nature 1998;393:635–6.

Jain S, Shukla SD, Sharma K, Bhatnagar M. Neuroprotective effects of *Withania somnifera* Dunn. in hippocampal sub-regions of female albino rat. Phytother Res 2001;15:544–8.

Kamei H, Nagai T, Nakano H, Togan Y, Takayanagi M, Takahashi K, et al. Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK1/2 activation in the prefrontal cortex of mice. Biol Psychiatry 2006;59:75–84.

Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. Science's STKE 2001;294(5544):1030–8.

Khalil M, Alam N, Moniruzzaman M, Sulaiman S, Gan S. Phenolic acid composition and antioxidant properties of Malaysian honeys. J Food Sci 2011;76:C921–8.

Kishore RK, Halim AS, Syazana MS, Sirajudeen KN. Tualang honey has higher phenolic content and greater radical scaveng-

ing activity compared with other honey sources. Nutr Res 2011;31:322–5.

Klintsova A, Levy WB, Desmond NL. Astrocytic volume fluctuates in the hippocampal CA1 region across the estrous cycle. Brain Res 1995;690:269–74.

Komuro E, Takahashi M, Morita T, Tsuchiya J, Arakawa Y, Yamamoto Y, et al. Inhibition of peroxidations of lipids and membranes by estrogens. J Phys Org Chem 1990;3:309–15.

Lacey JV Jr, Mink PJ, Lubin JH, Sherman ME, Troisi R, Hartge P, et al. Menopausal hormone replacement therapy and risk of ovarian cancer. JAMA 2002;288:334–41.

Leuner B, Mendolia-Loffredo S, Shors T. High levels of estrogen enhance associative memory formation in ovariectomized females. Psychoneuroendocrinology 2004;29:883–90.

Li C, Brake WG, Romeo RD, Dunlop JC, Gordon M, Buzescu R, et al. Estrogen alters hippocampal dendritic spine shape and enhances synaptic protein immunoreactivity and spatial memory in female mice. Proc Natl Acad Sci U S A 2004;101:2185–90.

Lu YP, Zeng M, Hu XY, Xu H, Swaab DF, Ravid R, et al. Estrogen receptor [alpha]-immunoreactive astrocytes are increased in the hippocampus in Alzheimer's disease. Exp Neurol 2003;183:482–8.

Luine V. Sex steroids and cognitive function. J Neuroendocrinol 2008;20:866–72.

Luine VN. Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. Exp Neurol 1985;89:484–90.

Luine VN, Jacome LF, MacLusky NJ. Rapid enhancement of visual and place memory by estrogens in rats. Endocrinology 2003;144:2836–44.

Lunny CA, Fraser SN. The use of complementary and alternative medicines among a sample of Canadian menopausal-aged women. J Midwifery Womens Health 2010;55:335–43.

MacLennan A, Lester S, Moore V. Oral estrogen replacement therapy versus placebo for hot flushes: a systematic review. Climacteric 2001;4:58–74.

Marder K, Sano M. Estrogen to treat Alzheimer's disease: Too little, too late? So what'sa woman to do? Neurology 2000;54:2035–7.

Markham JA, Greenough WT. Experience-driven brain plasticity: beyond the synapse. Neuron Glia Biol 2004;1:351–63.

McEwen BS, Sapolsky RM. Stress and cognitive function. Curr Opin Neurobiol 1995;5:205–16.

McEwen BS. Effects of adverse experiences for brain structure and function. Biol Psychiatry 2000;48:721–31.

McGaugh JL. Time-dependent processes in memory storage. Science 1966;153:1351–8.

Medina JH, Bekinschtein P, Cammarota M, Izquierdo I. Do memories consolidate to persist or do they persist to consolidate? Behav Brain Res 2008;192:61–9.

Miller J, Chan BK, Nelson HD. Postmenopausal estrogen replacement and risk for venous thromboembolism: a systematic review and meta-analysis for the U.S. Preventive Services Task Force. Ann Intern Med 2002;136:680–90.

Milner TA, Ayoola K, Drake CT, Herrick SP, Tabori NE, McEwen BS, et al. Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. Comp Neurol 2005;491:81–95.

Milner TA, McEwen BS, Hayashi S, Li CJ, Reagan LP, Alves SE. Ultrastructural evidence that hippocampal alpha estrogen receptors are located at extranuclear sites. J Comp Neurol 2001;429:355–71.

Mohamed M, Sirajudeen KNS, Swamy M, Yaacob NS, Sulaiman SA. Studies on the antioxidant properties of Tualang honey of Malaysia. AJTCAM Compl Med 2010;7:59–63.

Mukai K, Daifuku K, Yokohama S, Minoru N. Stopped-flow investigation of antioxidant activity of estrogens in solution. Biochim Biophys Acta (BBA): Gen Subjects 1990;1035:348–52.

Nagai T, Inoue R, Kanamori N, Suzuki N, Nagashima T. Characterization of honey from different floral sources. Its functional properties and effects of honey species on storage of meat. Food Chem 2006;15:256–62.

Ohl F, Fuchs E. Differential effects of chronic stress on memory processes in the tree shrew. Cogn Brain Res 1999;7:379–87.

Olton DS, Wible CG, Pang K, Sakurai Y. Hippocampal cells have mnemonic correlates as well as spatial ones. Psychobiology 1989;17:228–9.

Othman Z, Shafin N, Zakaria R, Hussain NHN, Mohammad WMZW. Improvement in immediate memory after 16 weeks of Tualang honey (Agro Mas) supplement in healthy postmenopausal women. Menopause 2011;18:1219–24.

Perez RA, Iglesias MT, Pueyo E, Gonzalez M, De Lorenzo C. Amino acid composition and antioxidant capacity of Spanish honeys. J Agric Food Chem 2007;55:360–5.

Pieta Dias C, Martins de Lima M, Presti-Torres J, Dornelles A, Garcia V, Siciliani Scalco F, et al. Memantine reduces oxidative damage and enhances long-term recognition memory in aged rats. Neuroscience 2007;146:1719–25.

Pilati N, Barker M, Panteleimonitis S, Donga R, Hamann M. A rapid method combining Golgi and Nissl staining to study neuronal morphology and cytoarchitecture. J Histochem Cytochem 2008;56:539–50.

Regan MM, Emond SK, Attardo MJ, Parker RA, Greenspan SL. Why do older women discontinue hormone replacement therapy? J Womens Health Gend Based Med 2001;10:343–50.

Reneerkens OAH, Rutten K, Akkerman S, Blokland A, Shaffer CL, Menniti FS, et al. Phosphodiesterase type 5 (PDE5) inhibition improves object recognition memory: Indications for central and peripheral mechanisms. Neurobiol Learn Mem 2012;97:370–9.

Resnick SM, Maki PM, Rapp SR, Espeland MA, Brunner R, Coker LH, et al. Effects of combination estrogen plus progestin hormone treatment on cognition and affect. J Clin Endocrinol Metab 2006;91:1802–10.

Rozenberg S, Vandromme J, Antoine C. Postmenopausal hormone therapy: risks and benefits. Nat Rev Endocrinol 2013;9:216–27.

Sapolsky RM. Do glucocorticoid concentrations rise with age in the rat? Neurobiol Aging 1992;13:171–4.

Scharfman HE, Hintz TM, Gomez J, Stormes KA, Barouk S, Malthankar-Phatak GH, et al. Changes in hippocampal function of ovariectomized rats after sequential low doses of estradiol to simulate the preovulatory estrogen surge. Eur J Neurosci 2007;26:2595–612.

Sherwin BB, Henry JF. Brain aging modulates the neuroprotective effects of estrogen on selective aspects of cognition in women: a critical review. Front Neuroendocrinol 2008;29:88–113.

Shughrue P, Merchenthaler I. Evidence for novel estrogen binding sites in the rat hippocampus. Neuroscience 2000;99:605–12.

Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, et al. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women. JAMA 2004;291:2947–58.

Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, et al. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 2003;289:2651–62.

Singer CA, Figueroa-Masot XA, Batchelor RH, Dorsa DM. The mitogen-activated protein kinase pathway mediates estrogen

neuroprotection after glutamate toxicity in primary cortical neurons. J Neurosci 1999;19:2455–63.

Singh M, Sétáló G Jr, Guan X, Warren M, Toran-Allerand CD. Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. J Neurosci 1999;19: 1179–88.

Su J, Sripanidkulchai K, Hu Y, Wyss JM, Sripanidkulchai B. The effect of ovariectomy on learning and memory and relationship to changes in brain volume and neuronal density. Int J Neurosci 2012;122:549–59.

Takuma K, Matsuo A, Himeno Y, Hoshina Y, Ohno Y, Funatsu Y, et al. 17β-Estradiol attenuates hippocampal neuronal loss and cognitive dysfunction induced by chronic restraint stress in ovariectomized rats. Neuroscience 2007;146:60–8.

Tang Y, Haslam S, Conrad S, Sisk C. Estrogen increases brain expression of the mRNA encoding transthyretin, an amyloid beta scavenger protein. J Alzheimers Dis 2004;6:413–20.

Tierney MC, Oh P, Moineddin R, Greenblatt EM, Snow WG, Fisher RH, et al. A randomized double-blind trial of the effects of hormone therapy on delayed verbal recall in older women. Psychoneuroendocrinology 2009;34:1065–74.

Vedder H, Anthes N, Stumm G, Würz C, Behl C, Krieg JC. Estrogen hormones reduce lipid peroxidation in cells and tissues of the central nervous system. J Neurochem 1999;72:2531–8.

Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Suissa S, Horwitz RI. A clinical trial of estrogen-replacement therapy after ischemic stroke. N Engl J Med 2001;345:1243–9.

Walf AA, Rhodes ME, Frye CA. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol Learn Mem 2006;86:35–46.

Wallace M, Luine V, Arellanos A, Frankfurt M. Ovariectomized rats show decreased recognition memory and spine density in the hippocampus and prefrontal cortex. Brain Res 2006;1126:176–82.

Warden MR, Miller EK. Task-dependent changes in short-term memory in the prefrontal cortex. J Neurosci 2010;30:15801–10.

Wiegert O, Joëls M, Krugers H. Timing is essential for rapid effects of corticosterone on synaptic potentiation in the mouse hippocampus. Learn Mem 2006;13:110–3.

Woolley CS, Schwartzkroin PA. Hormonal effects on the brain. Epilepsia 1998;39:S2–8.

Yamada K, Takayanagi M, Kamei H, Nagai T, Dohniwa M, Kobayashi K, et al. Effects of memantine and donepezil on amyloid beta-induced memory impairment in a delayed-matching to position task in rats. Behav Brain Res 2005;162:191–9.

Yu X, Rajala RV, McGinnis JF, Li F, Anderson RE, Yan X, et al. Involvement of insulin/phosphoinositide 3-kinase/Akt signal pathway in 17 beta-estradiol-mediated neuroprotection. J Biol Chem 2004;279:13086–94.

Zaid SS, Sulaiman SA, Sirajudeen KN, Othman NH. The effects of Tualang honey on female reproductive organs, tibia bone and hormonal profile in ovariectomised rats – animal model for menopause. BMC Complement Altern Med 2010;31(10):82.

Zhang J, Inazau M, Tsuji K, Yamada E, Takeda H, Matsumiya T. Neurochemical characteristics and behavioral responses to psychological stress in ovariectomized rats. Pharmacol Res 1999;39:455–61.

Zhang YQ, Shi J, Rajakumar G, Day AL, Simpkins JW. Effects of gender and estradiol treatment on focal brain ischemia. Brain Res 1998;784:321–4.

Zhao L, Wu T-W, Brinton RD. Estrogen receptor subtypes alpha and beta contribute to neuroprotection and increased Bcl-2 expression in primary hippocampal neurons. Brain Res 2004;1010:22–34.