EVALUATION OF EFFECTS OF EXPERIMENTAL EXOGENOUS FEVER, HYPERTHERMIA AND VARIOUS DRUGS ON INFANT RAT BRAIN

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ABSTRACT

Objective: *Hyperthermia may cause pathological changes in all systems and organs including the brain. Neuronal effects of exogenous fever (39°C) and hyperthermia (41°C), and efficacy of different medication modalities were studied in two-week-old infant female Wistar-Albino rats.*

Material and Method: *Possible neuronal damage was evaluated by examining healthy, apoptotic and necrotic cells, and heat shock proteins (HSP, HSP 27 and HSP 70) in the cerebral cortex, cerebellum, and hypothalamus.*

Results: *In both temperature groups, convulsion has been observed at different rates (25-37.5%). Three infant rats with convulsion in the group of 41 °C temperature were died (n=3/9, 33.3%). At cellular level, when all neural tissues were taken into account; (i) considerable increase in number of necrotic neurons in both temperature groups (p=0.001, p=0.000), (ii) after 39°C fever, a decrease in number of healthy cells by diclofenac medication (p=0.02), an increase in number of necrotic cells by dexamethasone (p=0.02) and diclofenac (p=0.005) medications, (iii) after 41°C hyperthermia, a decrease in number of necrotic cells by dexamethasone (p=0.000) and paracetamol medications (p=0.000) were observed. In the group of 39°C fever, all medications were ineffective in terms of the number of apoptotic cells (p>0.05).*

Conclusion: *In conclusion, results of the present study showed that neuronal tissue of various brain regions responded as different degree of damage or improvement to hyperthermic time course and applied medications. It was considered that these conflicting data might be due to the complexity of the brain.*

Key Words: *Fever, hyperthermia, brain, neurons* ***Nobel Med 2012; 8(3): 66-75***

DENEYSEL OLARAK OLUŞTURULMUŞ EKZOJEN ATEŞ, HİPERTERMİ VE BUNA YÖNELİK KULLANILAN BAZI İLAÇLARIN YAVRU RAT BEYNİ ÜZERİNE OLAN ETKİLERİNİN DEĞERLENDİRİLMESİ

ÖZET

Amaç: Hipertermi, beyin dahil tüm organlar ve sistemler üzerinde patolojik değişikliklere neden olabilmektedir. Bu çalışmada, ekzojen yolla oluşturulmuş ateş (39°C) ve hiperterminin (41°C) nöronal etkileri ile değişik tedavi yöntemlerinin etkinlikleri iki haftalık Wistar-Albino cinsi dişi yavru ratlarda araştırıldı.

Materyal ve Metod: Olası nöronal hasar; serebral korteks, serebellum ve hipotalamustaki sağlam, nekrotik ve apoptotik hücreler ile birlikte ısı şok proteinleri (Heat Shock Protein=HSP, HSP 27 ve HSP 70) incelenerek değerlendirildi.

Bulgular: Her iki sıcaklık grubunda farklı oranlarda (%25-37,5) nöbet gözlendi. 41°C ısı grubunda nöbet geçiren üç yavru ratın (n=3/9, %33,3) öldüğü görüldü. Tüm nöral dokular dikkate alındığı zaman hücresel seviyede; (i) her iki sıcaklık grubunda da nekrotik nöron sayısında anlamlı bir artış (p=0,001, p=0,000), (ii) 39°C ateş sonrasında diklofenak tedavisi ile sağlam hücre sayısında azalma (p=0,02), deksametazon ve diklofenak tedavisi ile nekrotik hücre sayısında artış (sırasıyla p=0,02 ve p=0,005), (iii) 41°C hipertermi sonrasında ise deksametazon ve parasetamol tedavileri ile nekrotik hücre sayısında azalma (sırasıyla, p=0,000 ve p=0,000) gözlendi. 39°C ateş grubunda tüm tedavi yöntemlerinin apoptotik hücre sayısı üzerine herhangi bir etkisinin olmadığı görüldü (p>0,05).

Sonuç: Bu çalışmanın sonuçları, beynin farklı bölgelerindeki nöronal dokunun hipertermik sürece ve uygulanan medikasyonlara değişik düzeylerde hasar veya iyileşme yanıtları verdiğini göstermektedir. Elde ettiğimiz bu farklı sonuçların beynin kompleks yapısına bağlı olabileceği düşünüldü.

Anahtar Kelimeler: Ateş, hipertermi, beyin, nöronlar
Nobel Med 2012; 8(3): 66-75

INTRODUCTION

Fever is one of the most frequently observed symptom as well as a major patient complaint in the pediatric practice. Child brain can experience several pathological conditions including seizure even at low fever levels.[1,2] Due to obvious difficulties to perform studies to explore the effects of high fever and hyperthermia on the brains of children, studies are limited to experiments utilizing animal models. There are studies carried out using rat model which reporting the effects of hypothermia and hyperthermia resulting in brain damage.[3-6] Hyperthermia may cause changes in whole body systems and organs. It has been reported that deficiency in learning process and memory loss, adaptation and behavioral problems and problems related to motor activity may be due to the effects of hyperthermia on central nervous system.[7-9] Hyperthermia has been reported to cause apoptosis. In addition, increased apoptotic process continues during hyperthermia. Apoptotic process is affected by duration of hyperthermia until treatment is initiated.[10,11] Recent studies indicate that rate of cell deaths observed in post-ischemic phase may increase or decrease depending on heat.[12]

It has been well documented that increase in temperature may result fatigue, thermogenic anhydrous, heat stroke and even death. Cerebral edema, bleeding regions in cerebrum and cerebellum, development of neuronal degenerations in cerebral and cerebellar cortexes have been observed in postmortem investigations of exogenous hyperthermia related deaths. Changes in cerebellum develop faster than the ones in other parts of brain. They are more prominent in cerebellum while purkinje cell numbers are decreased and remaining ones are necrotic.[6,8] Although there are studies investigating the effects of endogenous hyperthermia on cerebrum, studies focusing on the effects of experimental exogenous hyperthermia on neuronal changes are limited.[4] The present study aimed to deciphering the neuronal effects of experimentally induced exogenous fever and hyperthermia and effects of various medications on possible damages due to fever and hyperthermia.

MATERIAL and METHOD

Animals and experimental groups

Total of 54 infant (two-weeks-old) female Wistar-Albino rats (55±10 gr) were used in the study. After approval of study by animal care committee, the rats were housed in a temperature controlled room (22-25°C) with a 12:12 light-dark cycle; water and food were given *ad libitum*. Three groups were generated by distributing animals randomly as described below. Animals in group I ("fever group" with rectal temperature of 39°C; n=24), and group II ("hyperthermia group" with rectal temperature of 41°C; n=24) were exposed to heat stress as described earlier.[4,13] Group III ("control group", n=6) →

Table 1: Effects of fever and hyperthermia induced damages on healthy and necrotic cell numbers

Study Groups (39°C/41°C)	Brain Region	Healthy Cell Number*	Necrotic Cell Number*
Saline administered group	Cerebral cortex	Increase / Increase	Unvaried / Unvaried
	Cerebellum	Decrease / Unvaried	Unvaried / Increase
	Hypothalamus	Decrease / Decrease	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried	Increase / Increase
Dexamethasone administered group	Cerebral cortex	Unvaried / Decrease	Unvaried / Unvaried
	Cerebellum	Unvaried / Unvaried	Decrease / Decrease
	Hypothalamus	Decrease / Increase	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried	Increase / Decrease
Paracetamol administered group	Cerebral Cortex	Unvaried / Decrease	Unvaried / Decrease
	Cerebellum	Unvaried / Unvaried	Decrease / Decrease
	Hypothalamus	Unvaried / Increase	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried	Unvaried / Decrease
Diclofenac administered group	Cerebral Cortex	Unvaried / Unvaried	Increase / Unvaried
	Cerebellum	Unvaried / Decrease	Decrease / Decrease
	Hypothalamus	Decrease / Increase	Increase / Unvaried
	All neuronal regions	Decrease / Unvaried	Increase / Unvaried

*Increase/Decrease: $p<0.05$; unvaried: $p>0.05$

was composed of animals which were not exposed to neither fever nor hyperthermia and kept under normal conditions.

Animals in groups I and II were further divided into 4 subgroups: saline group (n=6), dexamethasone administered group (n=6), paracetamol administered group (n=6) and diclofenac administered group (n=6). Saline (0.5 ml) was administered via intraperitoneal (*i.p.*) route every 6 hr for 5 days as described earlier.[14] Dexamethasone (200 µg/kg) diluted in 0.5 ml saline was injected (*i.p*) every 6 hr for 5 days.[15] Paracetamol (15 mg/kg) was administered via orogastric route every 6 hr for 5 days.[16] Diclofenac (10 mg/kg) was administered (*i.p.*) every 6 hr for 5 days.[17]

Induction of fever or hyperthermia

Hyperthermia Induction Chamber (HIC) was designed by Drs. Bayram Yılmaz and Özgür Bulmuş HIC with dimensions of 40x40x35 cm was constructed using clear glass. Two holes were created for air flow on the two opposing walls of the chamber. Lid was located on the top of the chamber for easy access. An adjustable heat regulator was located inside the lid. Two digital thermometers were located on inner surface of two walls facing each other allowing measurement of heat from outside. Animals were placed in the HIC in groups (n=6) allowing to be exposed exogenous heat stress. Before placing a new group of animals, chamber was cleaned and its temperature was checked and adjusted as desired. While temperature was set to 48°C, temperature inside the HIC was 45°C. Rectal temperatures of animals were monitor via using rectal probes as described earlier.[4] When desired temperature as fever or hyperthermia was achieved, animals were exposed to this constant temperature for 60 min.

Histopathological and immunohistochemical analysis

After five days of the end of the experimental procedures after five days, all animals were decapitated and brains were removed as quickly as possible. The brains were fixed in 10% formalin and embedded in paraffin. Coronal slices were obtained at cerebral cortex, hypothalamus and cerebellum according to a stereotaxic atlas of the developing rat brain. Neuropathological evaluation was performed using the hippocampus, cerebral cortex and cerebellum on the coronal plane.

After slices were obtained with microtome, samples were stained with hematoxylin and eosin (H&E). Stained preparations were examined under light microscopy (x400). Possible neurological damage findings were evaluated according to neuroanatomical regions. For this purpose, healthy and necrotic cells were counted at six different areas in cerebral cortex, cerebellum and hypothalamus. Subsequently means obtained from six different areas were used. Scores obtained from counting healthy and necrotic neurons and their ratio were used to assess brain damage.[18]

Sections 20 mm in thickness were obtained and stained with hematoxylin-eosin and with TdT-mediated dUTPbiotin nick-end labeling (TUNEL). For TUNEL staining and evaluation of apoptosis, paraffin-embedded brain sections were deparaffinized in two changes of xylene for 5 min each, then hydrated in 100, 95 and 70% ethanol. After incubation with 20 $mgml^{-1}$ proteinase K for 5 min, a modified terminal deoxynucleotidyl transferase-mediated UTP nick-end labeling (TUNEL) method was applied using an in situ apoptosis detection kit (ApopTag, Oncor, USA).[19] Briefly, this includes immersion in equilibration buffer for 10 min, application of terminal deoxynucleotidyl transferase (TdT) and dUTP-digoxigenin at 37°C for 1 h, followed by stop/wash buffer at 37°C for 30 min., incubation with antidigoxigenin for 30 min, and visualization with 0.05% diaminobenzidine tetrachloride and 0.02% H_2O_2 in 50 mM Tris-HCl buffer. Counterstaining was done with methyl green. Slices treated similarly but in the absence of TdT enzyme, digoxigenin-dUTP, or anti-digoxigenin antibody were included as negative controls. Apoptotic neurons were determined using the image analysis system (Zeiss Vision KS400 version 3.0) by systematically randomized sampling through a 100-Nikon oil-immersion lens. The percentage of apoptotic neurons was calculated from apoptotic neurons to all neuron ratio in all brain regions, and scored as follows: 0=no apoptotic cells, 1=less than 25%, 2=25-50%, 3=50% and over. →

Two heat shock proteins (HSP), HSP 27 and HSP 70, were used for evaluating damages in the same parts of brain regions. For these purpose, HSP 27 and HSP 70 antibodies purchased from Lab Vision (Fremont, CA, USA) were used for in situ hybridizations. Obtained slices were marked with HSP 27 Ab-1 (Clone G3.1, Labvision, USA) and HSP 70 Ab-3 (Labvision, USA) antibodies.

Statistical Analysis

Statistical analysis was carried out by using the SPSS for Windows, ver. 12 (SPSS Inc., Chicago, IL, USA). The data obtained are expressed as mean±SD. Two-way ANOVA test was used for comparison among the groups. $p<0.05$ was considered to be statistically significant.

RESULTS

Behavioral changes observed in animals

Initially there was an increase in the movements of rats due to exposure to heat. Rats were climbing upon each other, laying down on their backs or sides, or they were trying to stand up in their back feet. Later on movements slowed down. We have observed generalized convulsions as tremors in extremities and lying down on sides in 6 animals (out of 24, 25%) with rectal temperature of 39°C and 9 animals (out of 24, 37.5%) with rectal temperature of 41°C. Three of the rats having convulsions with rectal temperature of 41°C (33.3%) died on second and third days. These rats were replaced with new animals.

Histopathological and immunohistochemical findings

Changes caused by damages due to fever and hyperthermia are presented in Table 1, 2, and 3 based on neuroanatomic regions. Statistically significant findings are summarized below:

1. Cerebral cortex

Effects of fever and hyperthermia on cerebral cortex

- We did not observe any significant changes in necrotic cell number at both temperatures ($p>0.05$). There was a statistically significant increase in healthy cell number of cerebral cortex at both temperatures ($p=0.006$ for 39°C, $p=0.004$ for 41°C) (Table 1).

- HSP 27 levels were not changed in fever and hyperthermia groups ($p>0.05$). HSP 70 levels were decreased at 39°C ($p=0.02$) whereas we did not observe a statistically significant change at 41°C ($p>0.05$) (Table 2).

Table 2: Effects of fever and hyperthermia induced damages on HSP 27 and HSP 70 levels

Study Groups (39°C/41°C)	Brain Region	HSP 27 levels*	HSP 70 levels *
Saline administered group	Cerebral Cortex	Unvaried / Unvaried	Decrease / Unvaried
	Cerebellum	Unvaried / Increase	Unvaried / Increase
	Hypothalamus	Decrease / Unvaried	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried	Unvaried / Decrease
Dexamethasone administered group	Cerebral Cortex	Increase / Increase	Unvaried / Unvaried
	Cerebellum	Unvaried / Unvaried	Unvaried / Unvaried
	Hypothalamus	Unvaried / Decrease	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried	Unvaried / Unvaried
Paracetamol administered group	Cerebral Cortex	Unvaried / Unvaried	Decrease / Unvaried
	Cerebellum	Increase / Decrease	Unvaried / Increase
	Hypothalamus	Unvaried / Decrease	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried	Unvaried / Decrease
Diclofenac administered group	Cerebral Cortex	Unvaried / Increase	Increase / Decrease
	Cerebellum	Increase / Decrease	Unvaried / Increase
	Hypothalamus	Increase / Decrease	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried	Unvaried / Increase

*Increase/Decrease: $p<0.05$; unvaried: $p>0.05$, HSP: Heat shock protein

- There were significant increases in cerebral cortex apoptotic cell numbers in both temperature groups ($p=0.02$), (Table 3).

Effects of drug administrations on cerebral cortex

- There was no statistically significant changes in healthy cell numbers in cerebral cortex of animals with rectal temperature of 39°C upon treatment with the drugs ($p>0.05$). Necrotic cell number was increased with diclofenac ($p=0.004$) while other two drugs had no effect on necrotic cell number in cerebral cortex of animals with rectal temperature of 39°C ($p>0.05$) (Table 1). In hyperthermia group, dexamethasone and paracetamol caused significant reduction of healthy cell numbers ($p=0.04$, $p=0.005$, respectively) while diclofenac treatment did not cause any change ($p>0.05$) (Table 1). Paracetamol reduced necrotic cell number in hyperthermia group ($p=0.001$) (Table 1).

- Dexamethasone increased HSP 27 levels in 39°C fever group ($p=0.002$) whereas diclofenac and paracetamol had no effect ($p>0.05$) (Table 2). In hyperthermia group, HSP 27 levels were determined to be increased upon treatment with dexamethasone or diclofenac ($p=0.02$) while paracetamol had no effect ($p>0.05$) (Table 2). HSP 70 levels in 39°C fever group was increased with diclofenac while paracetamol caused a reduction ($p=0.02$). Dexamethasone did not affect HSP 70 levels in fever group ($p>0.05$) (Table 2). Treatment with diclofenac reduced HSP 70 levels in hyperthermia group while other two drugs had no effect ($p>0.05$) (Table 2).

- All three drugs had no effect on apoptosis in 39°C→

Table 3: Effects of fever and hyperthermia induced damages on neuronal apoptotic process

Study Groups (39°C/41°C)	Brain Region	Apoptotic cell levels*
Saline administered group	Cerebral Cortex	Increase / Increase
	Cerebellum	Unvaried / Increase
	Hypothalamus	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried
Dexamethasone administered group	Cerebral Cortex	Unvaried / Increase
	Cerebellum	Increase / Unvaried
	Hypothalamus	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried
Paracetamol administered group	Cerebral Cortex	Unvaried / Increase
	Cerebellum	Increase / Unvaried
	Hypothalamus	Unvaried / Unvaried
	All neuronal regions	Unvaried / Unvaried
Diclofenac administered group	Cerebral Cortex	Unvaried / Increase
	Cerebellum	Unvaried / Decrease
	Hypothalamus	Unvaried / Unvaried
	All neuronal regions	Unvaried / Decrease

*Increase/Decrease: $p<0.05$; unvaried: $p>0.05$

fever group ($p>0.05$) while they increased apoptotic cell numbers in 41°C hyperthermia group ($p=0.02$) (Table 3).

2. Cerebellum

Effects of fever and hyperthermia on cerebellum

- There was a significant reduction in healthy neuron numbers of infant rats exposed to 39°C fever compared to the control ones ($p=0.05$) while necrotic cell numbers were increased in infant animals exposed to 41°C hyperthermia ($p=0.01$) (Table 1).

- HSP 27 and HSP 70 levels were unchanged in 39°C fever group while 41°C hyperthermia increased both HSP levels significantly ($p=0.02$) (Table 2).

- While 39°C fever did not effect apoptotic process ($p>0.05$), 41°C hyperthermia caused a significant increase ($p=0.02$) (Table 3).

Representative histopathological and immuno-histochemical images from cerebellums of rats exposed to hyperthermia and received only saline those are perivascular edema (A), necrosis (B), HSP 27 + cells (C), HSP 70 + cells (D), and apoptotic cells (E) are shown in Figure 1.

Effects of drug administrations on cerebellum

- All three drugs used in the study did not cause any change in healthy cell numbers ($p>0.05$) while they decreased necrotic cell numbers significantly in 39°C fever group ($p=0.004$) (Table 1). In 41°C hyperthermia group, diclofenac reduced healthy cell number ($p=0.002$) while dexamethasone and paracetomol had no significant effect. All three drugs reduced necrotic cell numbers significantly in hyperthermia group ($p=0.004$) (Table 1).

- While diclofenac and paracetamol increased HSP 27 levels in 39°C fever group ($p=0.02$), same drugs caused a reduction of HSP 27 levels in 41°C hyperthermia group ($p=0.04$). On the other hand, HSP 70 levels were not affected in 39°C fever group by any of the drugs ($p>0.05$). Paracetamol and diclofenac caused a significant increase in HSP 70 levels in 41°C hyperthermia group ($p=0.02$) (Table 2).

- Dexamethasone and paracetamol applications in 39°C fever group resulted in increase of apoptotic process ($p=0.02$) while diclofenac reduced this process significantly in 41°C hyperthermia group ($p=0.02$) (Table 3).

3. Hypothalamus

Effects of fever and hyperthermia on hypothalamus

- There was a statistically significant reduction in healthy neuron numbers in both 39°C fever and 41°C hyperthermia groups ($p=0.02$ and $p=0.04$, respectively) while necrotic cell numbers were not changed significantly ($p>0.05$) (Table 1).

- HSP 27 levels were decreased significantly in 39°C fever group ($p=0.02$) while remained unchanged in 41°C hyperthermia group ($p>0.05$) (Table 2). We did not observe any significant change in HSP 70 levels in both temperature groups ($p>0.05$) (Table 2).

- There was not any changes in apoptotic processes on hypothalamic tissues by neither 39°C nor 41°C heat stress ($p>0.05$) (Table 3).

Effects of drug administrations on hypothalamus

- There was a significant reduction in healthy cell number in 39°C fever group due to dexamethasone and diclofenac administration ($p=0.01$ and $p=0.006$, respectively) (Table 1). In addition, we observed that diclofenac also increased necrotic cell numbers. All three drugs caused significant increases in healthy cell numbers in 41°C hyperthermia group ($p=0.004$, $p=0.05$, and $p=0.004$, respectively) while none of them changed necrotic cell numbers significantly ($p>0.05$) (Table 1).

- While only diclofenac administration caused a statistically significant increase in HSP 27 levels in 39°C fever group ($p=0.02$), all three drugs reduced HSP 27 levels in 41°C hyperthermia group ($p=0.02$) (Table 2).→

None of the drugs caused a significant change in HSP 70 levels in both 39°C and 41°C temperature groups (p>0.05) (Table 2).

4. Evaluation of all neuronal regions (cerebral cortex, cerebellum and hypothalamus)

Effects of fever and hyperthermia on all neuronal regions

- Although there was a significant increase in necrotic neuron numbers in both 39°C and 41°C temperature groups (p=0.001 and p=0.000, respectively), there was statistically insignificant change in healthy neuron numbers (p>0.05) (Table 1) (Figure 2A and 2B).

- There was no statistically significant change in HSP 27 levels in both 39°C and 41°C temperature groups (p>0.05). HSP 70 levels decreased in 41°C hyperthermia group (p=0.05) while there was no statistically significant changes in 39°C fever group (p>0.05) (Table 2) (Figure 2C, 2D).

- Neither 39°C fever nor 41°C hyperthermia had any significant effect on apoptotic process (p>0.05) (Table 3) (Figure 2E).

Effects of drug administrations on all neuronal regions

- In 39°C fever group diclofenac caused a reduction in healthy cell number (p=0.02) while necrotic cell numbers were elevated by diclofenac and dexamethasone administrations (p=0.005). We determined that necrotic cell numbers were reduced by dexamethasone and parasetamol in 41°C hyperthermia group (p=0.000) (Table 1).

- HSP 70 levels increased by diclofenac while paracetamol decreased the levels of this HSP in 41°C hyperthermia group (p=0.05) (Table 2).

- We observed a significant reduction in apoptotic cell numbers in 41°C hyperthermia group due to diclofenac administration (p=0.02) (Table 3).

DISCUSSION

Fever is one of the most frequently encountered finding and symptom in current pediatric practice. Moderate increase in fever may result in strong immune response following increase in production of interferons, lymphocyte transformation and leukocyte migration. On the other hand, immune response is suppressed by fever at 40°C and over. While metabolic effects of fever are tolerated by healthy children, in certain clinical cases these effects may result in dangerous scenarios. Increases of every 1°C in fever cause 10-12% increase in basal metabolism. As a result, oxygen consumption and carbon dioxide production are increased. Requirement for liquid and calorie intake are elevated. Febrile convulsions may be seen in predisposed children. Fever must be urgently treated symptomatically in children with tendency for febrile convulsion, clinically sick or suspected to have sepsis, with neurological damage or sickness, with metabolic problems or having fever 40°C and over.[1,2,12,20]

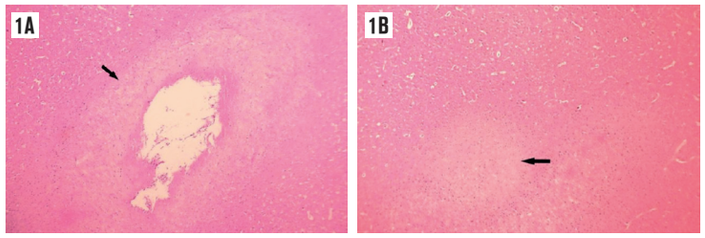

Figure 1A. Cerebellar section of a pup from hyperthermic (41°C) control group showing perivascular edema (H&E, x10). **Figure 1B.** Cerebellar section of a pup from hyperthermic (41°C) control group showing infarct (H&E, x40).

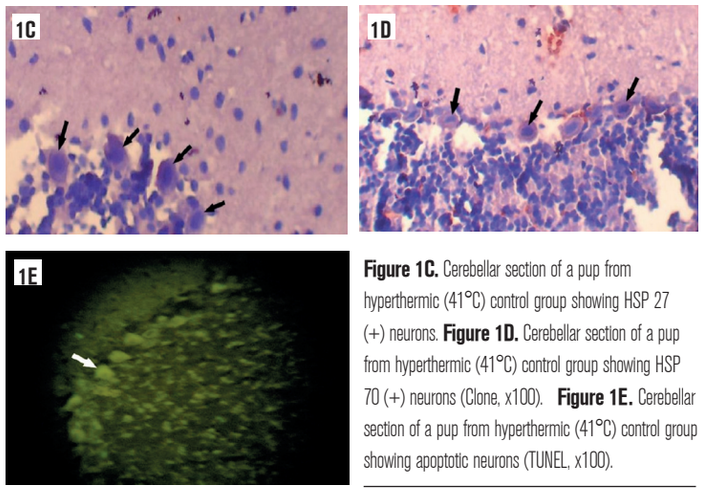

Figure 1C. Cerebellar section of a pup from hyperthermic (41°C) control group showing HSP 27 (+) neurons. **Figure 1D.** Cerebellar section of a pup from hyperthermic (41°C) control group showing HSP 70 (+) neurons (Clone, x100). **Figure 1E.** Cerebellar section of a pup from hyperthermic (41°C) control group showing apoptotic neurons (TUNEL, x100).

Although there are studies reporting results based on lipopolysaccharide-induced endogenous hyperthermia, there are limited number of studies exploring neuronal effects of experimentally-induced exogenous hyperthermia.[6,12,21] Earlier, Yager and Asselin evaluated the effect of pre-hypoxic-ischemic hypo- and hyperthermia on neuropathologic outcome in the immature rat brain.[4] Present study uniquely focused on evaluating effect of hyperthermia at 39°C and 41°C on possible neurological damage. Our study also examined effects of certain drugs on these processes at clinical and cellular levels. In addition, present study aimed to evaluate the effects of hyperthermic process at two different levels (39°C and 41°C).

As a result of hyperthermia-caused damages at cellular level, particularly at various parts of brain, blood brain barrier permeability is increased. Brain edema and damages in cells of several parts of brain are →

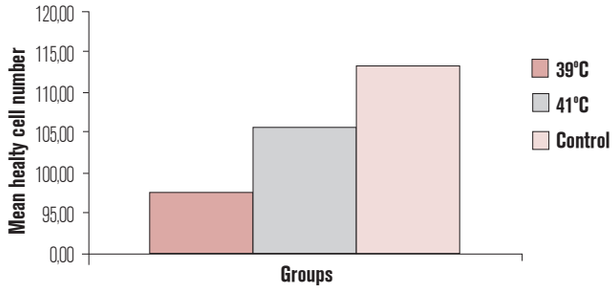

Figure 2A. Histopathological or immunohistochemical findings of all neural tissues examined in fever (39°C), hyperthermia (41°C) and control groups. Number of viable neurons ($p>0.05$)

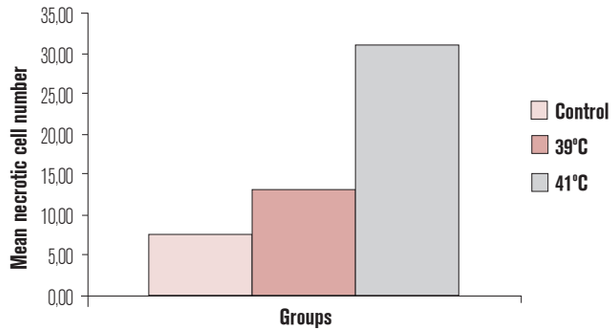

Figure 2B. Histopathological or immunohistochemical findings of all neural tissues examined in fever (39°C), hyperthermia (41°C) and control groups. Number of necrotic cells ($p<0.05$ for both groups)

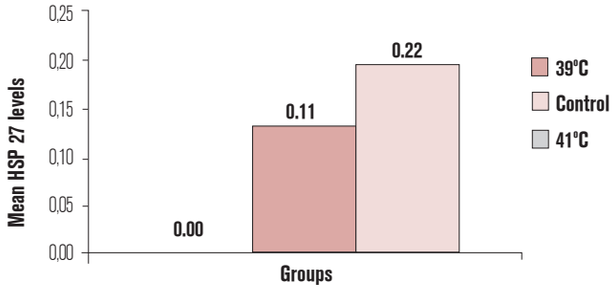

Figure 2C. Histopathological or immunohistochemical findings of all neural tissues examined in fever (39°C), hyperthermia (41°C) and control groups. HSP 27 percentages ($p>0.05$ for both groups)

developed.[22-24] Abraham et al. reported that hyperthermia may cause infarction areas in brain.[25] Different types of seizures due to hyperthermia have been reported by Ilbay et al.[26] In our study, clinical seizure was present in both 39°C and 41°C temperature groups, (25% and 37.5%, respectively). In addition, mortality rate was 33.3% in animals with convulsions. These observations clearly indicate the involvement of fever in etiology of seizures and subsequent convulsion-related mortalities. Similar to our findings, Trescher et al. reported that convulsion risk was 6% while mortality rate was 3% in their experimental study where they examined the effect of 38°C fever on rats.[1] Data from that study as well as data from our work indicate that gradual increase in fever results in clinically significant seizure and mortality.[1]

We have observed neuronal damaging process in different parts of the brain in both 39°C and 41°C temperature groups. Our results demonstrate that different drugs have positive effect against fever as well as some of these drugs may have side effects. These observations may be result of differential responses of different parts of neuronal tissues to hyperthermic process. Trescher et al. reported a mild damage (14.5%) caused by 38°C fever in cerebral cortex. On the other hand, Sinigaglia-Coimbra et al. reported a heavy damage (78.7%) in hippocampus region of rat brain caused by 38.5°C-40°C hyperthermia.[27]

In the present study, 39°C fever caused a reduction in healthy neurons in cerebellum and hypothalamus while there was an increase in the number of healthy neurons in cerebral cortex. These findings show that each of these three regions is affected differently by hyperthermic process. Cerebellar and hypothalamic structures appear to be more heat sensitive compare to cerebral cortex. In addition, we observed a continuation in reduction of healthy neuronal cell numbers of hypothalamus while necrotic cell numbers in cerebellum increased in 41°C hyperthermia group. Similar to the findings of the present study, other researchers such as Ekimova, and Khan and Brown also reported that hypothalamus and cerebellum are among the most heat sensitive regions of brain.[28,29]

Syntheses of HSPs, which are well known to be overproduced during stress, is increased in cells in which apoptotic process is induced following hyperthermia.[30,31] HSPs have roles in cellular regeneration and repairing cellular damages. Due to stress, particularly the production of HSP 70 is increased.[32-36] Kazanis et al. demonstrated in an experimental study that increased HSP 70 levels are protective for cellular structures.[7] In the present study, we did not observe any significant changes regarding HSP 27 and HSP 70 levels in the different parts of rat brains exposed to 39°C fever that caused brain damage. On the other hand, expressions of both of these HSPs are increased in cerebellum. Belay and Brown reported that levels of HSP are tended to increase in hyperthermically damaged rat brains, particularly in purkinje cells of cerebellum.[37] These findings may be an indicator of damage in cerebellum due to hyperthermia and subsequently developed cellular regeneration.

It has been well documented that hyperthermia inhibits cellular proliferation and increases apoptosis.[38-42] In the present study, we observed that both 39°C fever and 41°C hyperthermia caused an increase in apoptotic process in cerebral cortex while it was present only in cerebellum under exposure to 41°C hyperthermia. Singh demonstrated that cerebral cortex was among the regions where apoptotic changes seen following→

hyperthermic damage.[43] These results are in agreement with our findings indicating that apoptotic process may take place in cerebellum when temperature increases to 41°C.

In addition to evaluating the effects of fever and hyperthermia on different parts of brain, we extended our study by including drugs to examine their effects on the process. We have included three antipyretic drugs, namely, paracetamol, dexamethasone, and diclofenac. In addition to their anti-pyretic effects, dexamethasone and diclofenac have also strong anti-inflammatory and anti-edema features. Administration of all three drugs increased healthy neuron numbers significantly in hypothalamus of animals exposed 41°C hyperthermic damage. These drugs were also effective in reducing necrotic cell numbers in cerebellum in both 39°C and 41°C temperature groups. These results indicate that different medication models may be beneficial for temperature caused damage. Using drugs to protect brain regions which most severely affected by hyperthermia seem to be very advantageous based on our results and data from literature. Significant reduction in necrotic cell numbers in cerebral cortex due to administration of paracetamol during 41°C hyperthermia suggests that using these antipyretic drugs may be beneficial during hyperthermic process. However, there are reports with contradicting results.[16,44] Legos et al. reported that paracetamol or acetylsalicylic acid has no protective effect on cells of cerebral cortex or hypothalamus in their rat model exposed to 38°C and 39°C hyperthermic damage.[16] On the other hand, Sandrini et al. demonstrated that paracetamol and morphine combination was effective to reduce hyperthermic damage in rats exposed to 54°C room temperature for 15 seconds.[44]

It has been reported that HSP 27 and HSP 70 levels were elevated upon exposure to 42°C hyperthermia for 15 min. It has been proposed that hyperthermia-induced HSP 70 levels may be associated with pre-synaptic and post-synaptic elements. Particularly positioning of HSP 70 at the synapses may facilitate the repair of synaptic proteins which have been damaged by heat stress. In addition, presence of HSP 70 in synapses has been proposed to have neuroprotective effect.[45] In the present study, we demonstrated that antipyretic drugs have different effects on HSP 27 and HSP 70 levels in different parts of brain. For example, all three drugs elevated HSP 27 levels in any brain regions in 39°C fever group. On the other hand, administration of antipyretic drugs caused a reduction in HSP 27 levels in 41°C hyperthermia group in cerebellum and hypothalamus. We observed that HSP 70 levels in 39°C fever group were reduced in cerebral cortex while that were elevated in cerebellum of 41°C hyperthermia group following paracetamol and diclofenac administrations. These results clearly

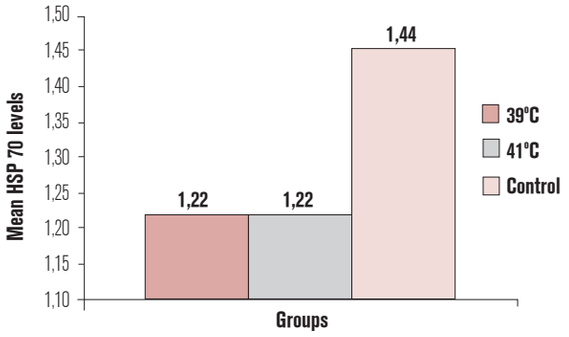

Figure 2D. Histopathological or immunohistochemical findings of all neural tissues examined in fever (39°C), hyperthermia (41°C) and control groups. HSP 70 percentages (39°C fever group: p>0.05 and 41°C hyperthermia group: p=0.05).

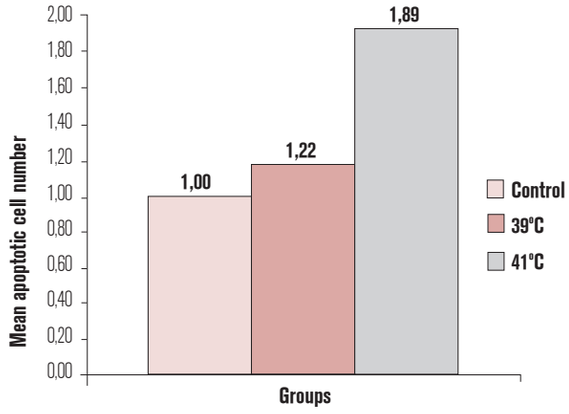

Figure 2E. Histopathological or immunohistochemical findings of all neural tissues examined in fever (39°C), hyperthermia (41°C) and control groups. Apoptotic cells (p>0.05 for both groups)

indicate the differential effects of hyperthermic damage and various drugs on HSP levels in different parts of brain. Lack of uniformity may be due to heterogenic cellular structures in neuronal tissues found in different parts of brain as well as different neurochemical and neurotransmitter contents.

Our findings regarding increase in apoptotic cell numbers in the cerebellum of the 39°C fever group due to administration of dexamethasone and paracetamol are in agreement with most of other study results. Drugs used in the present study either had no effect on hyperthermic process or had negative effects. Most of results from similar studies also show that medications are mainly efficient as antipyretic on hyperthermic process. For example, paracetamol alone reported to be not effective as a neuronal protector.[44] Different effects (positive, negative, or no effect) of drugs on hyperthermic process can be explained by complexity of brain where different neuronal structures and neuronal support structures are present. It has also been well documented that different parts of brain have different metabolism and neurotransmitter structures.[44-46] When all neuronal tissues were taken into account, we →

observed a significant increase in necrotic cell numbers during both heat stresses. This indicates that both fever and hyperthermia cause a significant loss of neurons. Lack of effects of fever and hyperthermia on apoptotic process and HSP levels suggests that apoptotic damage is not involved in early days of hyperthermic process. A report by Trescher et al. supported this hypothesis. They reported that hyperthermia causes an increase in apoptosis after the 4th week of hyperthermia.[1]

When all neuronal tissue was taken in account, we also determined that these medications were not beneficial. On the contrary, they may even be harmful. In 39°C fever group diclofenac reduced healthy cell numbers while dexamethasone and diclofenac increased necrotic cell numbers. Lack of beneficiary effects of all three medications in 41°C hyperthermic group indicate that they don't have any protective effect for healthy neurons. Nevertheless, dexamethasone and paracetamol administration reduced necrotic cell numbers in 41°C hyperthermia group suggesting that these drugs may have beneficiary effect during hyperthermic process. HSP 70 levels were reduced by paracetamol while diclofenac increased this HSP's levels in 41°C hyperthermia group.

CONCLUSION

In the present study, we demonstrate that fever and hyperthermia have detrimental effects on neuronal tissue of brain. In addition, we report that three widely used antipyretic drugs, namely paracetamol, dexamethasone and diclofenac, can have different effects on different neuronal structures during fever or hyperthermic process. These findings suggests that dexamethasone and diclofenac (which possess additional effects other than antipyretic effects) should not be used for treatment of fever or hyperthermia.

We realize that there are conflicting data in the present study. These conflicting data can be explained by complex structure of neuronal tissues of different brain regions. Heterogenic cellular structures with different neuronal and neurotransmitter contents make it more complex to analyze the effect of fever and hyperthermia as well as effects of certain drugs on overall process.

In addition, intravariability within the subject or subtype within the species that responds different to heat or drug insult, and the semi-quantitative methodology used in the present study might also be a source of discrepancy in the presented data. Further studies with a larger number of infant rats are required to compare and confirm present results. Researches like our study will allow us to develop new treatment modalities for clinical problems seen following exogenous hyperthermia.

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REFERENCES

1. Trescher WH, Ishiwa S, Johnston MV. Brief post-hypoxic-ischemic hypothermia markedly delays neonatal brain injury. Brain Dev 1997; 19: 326-338.
2. Mishima K, Ikeda T, Yoshikawa T, et al. Effects of hypothermia and hyperthermia on attentional and spatial learning deficits following neonatal hypoxia-ischemic insult in rats. Behav Brain Res 2004; 151: 209-217.
3. Daenen EW, Van der Heyden JA, Kruse CG, Wolterink G, Van Ree JM. Adaptation and habituation to an open field and responses to various stressful events in animals with neonatal lesions in the amygdala or ventral hippocampus. Brain Res 2001; 918: 153-165.
4. Yager JY, Asselin J. The effect of pre hypoxic-ischemic (HI) hypo and hyperthermia on brain damage in the immature rat. Brain Res Dev Brain Res 1999; 117: 139-143.
5. Nakai A, Shibazaki Y, Taniuchi Y, et al. Influence of mild hypothermia on delayed mitochondrial dysfunction after transient intrauterine ischemia in the immature rat brain. Brain Res Dev Brain Res 2001; 128: 1-7.
6. Chen GZ, Luo BD, Wang HQ, et al. Effects of hyperthermia on rat hippocampal pyramidal cell apoptosis in vitro. Di Yi Jun Yi Da Xue Xue Bao 2003; 23: 233-235.
7. Kazanis I, Bozas E, Philippidis H, Stylianopoulou F. Neuroprotective effects of insulin-like growth factor-I (IGF-I) following a penetrating brain injury in rats. Brain Res 2003; 991: 34-45.
8. Wada T, Kondoh T, Tamaki N. Ischemic "cross" tolerance in hypoxic ischemia of immature rat brain. Brain Res 1999; 847: 299-307.
9. Tomimatsu T, Fukuda H, Kanagawa T, et al. Effects of hyperthermia on hypoxic-ischemic brain damage in the immature rat: its influence on caspase-3-like protease. Am J Obstet Gynecol 2003; 188: 768-773.
10. Gilbert ME, Cain DP. A single neonatal pentylenetetrazol or hyperthermia convulsion increases kindling susceptibility in the adult rat. Brain Res 1985; 354: 169-180.
11. Morrison AJ, Rush SJ, Brown IR. Heat shock transcription factors and the hsp70 induction response in brain and kidney of the hyperthermic rat during postnatal development. J Neurochem 2000; 75: 363-372.
12. Heida JG, Boisse L, Pittman QJ. Lipopolysaccharide-induced febrile convulsions in the rat: short-term sequelae. Epilepsia 2004; 45: 1317-1329.
13. Mohamed F, Marchettini P, Stuart OA, Urano M, Sugarbaker PH. Thermal enhancement of new chemotherapeutic agents at moderate hyperthermia. Ann Surg Oncol 2003; 10: 463-468.
14. Pataki I, Jaszberenyi M, Telegdy G. Hyperthermic effect of centrally administered natriuretic peptides in the rat. Peptides 1999; 20: 193-197.
15. Ben-Hur T, Cialic R, Itzik A, et al. A novel permissive role for glucocorticoids in induction of febrile and behavioral signs of experimental herpes simplex virus encephalitis. Neuroscience 2001; 108: 119-127.
16. Legos JJ, Mangoni AA, Read SJ, et al. Programmable microchip monitoring of post-stroke pyrexia: effects of aspirin and paracetamol on temperature and infarct size in the rat. J Neurosci Methods 2002; 113: 159-166.
17. Seabra Mde L, Tufik S. Sodium diclofenac inhibits hyperthermia induced by paradoxical sleep deprivation: the possible participation of prostaglandins. Physiol Behav 1993; 54: 923-926.
18. Coimbra C, Boris-Möller F, Drake M, Wieloch T. Diminished neuronal damage in the rat brain by late treatment with the antipyretic drug

dipyrone or cooling following cerebral ischemia. Acta Neuropathol 1996; 92: 447-453.
19. Kabakus N, Ay I, Aysun S, et al. Protective effects of valproic acid against hypoxic-ischemic brain injury in neonatal rats. J Child Neurol 2005; 20: 582-587.
20. Aronoff DM, Neilson EG. Antipyretics: mechanisms of action and clinical use in fever suppression. Am J Med 2001; 111: 304-315.
21. Shibata M, Uno T, Riedel W, Nishimaki M, Watanabe K. Transiently enhanced LPS-induced fever following hyperthermic stress in rabbits. Int J Biometeorol 2005; 50: 67-74.
22. Sharma HS, Dey PK. Influence of long-term acute heat exposure on regional blood-brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. Brain Res 1987; 424: 153-162.
23. Sharma HS, Cervos-Navarro J. Brain oedema and cellular changes induced by acute heat stress in young rats. Acta Neurochir Suppl (Wien) 1990; 51: 383-386.
24. Sharma HS, Westman J, Nyberg F. Pathophysiology of brain edema and cell changes following hyperthermic brain injury. Prog Brain Res 1998; 115: 351-412.
25. Abraham H, Somogyvari-Vigh A, Maderdrut JL, Vigh S, Arimura A. Filament size influences temperature changes and brain damage following middle cerebral artery occlusion in rats. Exp Brain Res 2002; 142: 131-138.
26. Ilbay G, Sahin D, Ates N. Changes in blood-brain barrier permeability during hot water-induced seizures in rats. Neurol Sci 2003; 24: 232-235.
27. Sinigaglia-Coimbra R, Cavalheiro EA, Coimbra CG. Postischemic hyperthermia induces Alzheimer-like pathology in the rat brain. Acta Neuropathol 2002; 103: 444-452.
28. Ekimova IV. Changes in the metabolic activity of neurons in the anterior hypothalamic nuclei in rats during hyperthermia, fever, and hypothermia. Neurosci Behav Physiol 2003; 33: 455-460.
29. Khan VR, Brown IR. The effect of hyperthermia on the induction of cell death in brain, testis, and thymus of the adult and developing rat. Cell Stress Chaperones 2002; 7: 73-90.
30. Schlesinger MJ. Heat shock proteins. J Biol Chem 1990; 265: 12111-12114.
31. Mosser DD, Caron AW, Bourget L, et al. The chaperone function of hsp70 is required for protection against stress-induced apoptosis. Mol Cell Biol 2000; 20: 7146-7159.
32. Georgopoulos C, Welch WJ. Role of the major heat shock proteins as molecular chaperones. Annu Rev Cell Biol 1993; 9: 601-634.
33. Parsell DA, Lindquist S. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu Rev Genet 1993; 27: 437-496.
34. Hightower LE, Li T. Structure and Function of the Mammalian Hsp70 Family, Mayer J, Brown I. (eds). Heat Shock Proteins in the Nervous System. Academic Press, London 1994: 1-30.
35. Morimoto RI, Tissieres A, Georgopoulos C. The Biology of Heat Shock Proteins and Molecular Chaperones, New York, Cold Spring Harbor Laboratory Press. 1994.
36. Wu B, Hunt C, Morimoto R. Structure and expression of the human gene encoding major heat shock protein HSP70. Mol Cell Biol 1985; 5: 330-341.
37. Belay HT, Brown IR. Cell death and expression of heat-shock protein Hsc70 in the hyperthermic rat brain. J Neurochem 2006; 97: 116-119.
38. Kuwahara M. Thermosensitivity of glioma cells with special reference to changes in cytoskeletons. Neurol Med Chir (Tokyo) 1991; 31: 853-858.
39. Shinohara C, Matsumoto K, Maeda Y, et al. Synergistic effect of carboplatin and hyperthermia in rat and human glioma cell lines No Shinkei Geka 1994; 22: 1029-1033.
40. Watanabe M, Tanaka R, Hondo H, Kuroki M. Effects of antineoplastic agents and hyperthermia on cytotoxicity toward chronically hypoxic glioma cells. Int J Hyperthermia 1992; 8: 131-138.
41. Itoh N, Yonehara S, Ishii A, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 1991; 66: 233-243.
42. Yin D, Kondo S, Barnett GH, Morimura T, Takeuchi J. Tumor necrosis factor-alpha induces p53-dependent apoptosis in rat glioma cells. Neurosurgery 1995; 37: 758-763.
43. Singh NP. A simple method for accurate estimation of apoptotic cells. Exp Cell Res 2000; 256: 328-337.
44. Sandrini M, Vitale G, Ottani A, Pini LA. The potentiation of analgesic activity of paracetamol plus morphine involves the serotonergic system in rat brain. Inflamm Res 1999; 48: 120-127.
45. Bechtold DA, Rush SJ, Brown IR. Localization of the heat-shock protein Hsp70 to the synapse following hyperthermic stress in the brain. J Neurochem 2000; 74: 641-646.
46. Kiyatkin EA, Brown PL. Modulation of physiological brain hyperthermia by environmental temperature and impaired blood outflow in rats. Physiol Behav 2004; 83: 467-474.