

## Antioxidant Stobadine and Neurobehavioural Development of the Rat Offspring

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**Abstract.** Stobadine (STO) is a potential neuro- and cardioprotective drug with high antioxidative properties. The presented study investigated the effects of oral STO administration (5, 15 and 50 mg/kg/d) during pregnancy and lactation to dams on neurobehavioural development of their offspring (body growth and maturation, sensory functions, neuromotor and reflex development, levels of activity and emotional reactivity, memory and learning processes). The results of our experiments showed that long-term administration of STO had no adverse effects on the course of pregnancy and lactation in dams and on the neurobehavioural development of offspring.

**Key words:** Antioxidant — Stobadine — Development — Behaviour — Offspring — Rat

### Introduction

Oxidative stress represents a high risk for the developing organism, which is insufficiently protected by antioxidative enzyme systems (Sastre et al. 1994; Fantel 1996). From this point of view there is a growing need for effective prevention and treatment of diseases induced by oxidative stress. Natural and synthetic antioxidants have been used to advantage also in neonatology (Saugstad 1990; Allen and Venkatraj 1992; Tekulics et al. 1993).

Stobadine (STO), cis-(*-*)-2,3,4,4a,5,9b-hexahydro-2,8-dimethyl-1*H*-pyrido[4,3*b*]indole (CAS No. 95751-51-2), is a potential neuro- and cardioprotective drug with high antioxidative properties (Horáková and Štolc 1998). Extensive toxicological evaluation of STO on rats revealed no adverse effects (Gajdošíková et al. 1995). Similarly, results from reproductive toxicity (Balonová et al. 1991) and tera-

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tological studies in rats (Ujházy et al 1992) showed no teratogenic potential STO crosses the placental barrier in rats (Krištofová et al 1991) and rabbits (Ujházy et al 1999) and the blood-brain barrier in rats (Pavlásek et al 1996)

The presented study investigated the effect of STO administration during pregnancy and lactation to dams on neurobehavioural development of their offspring

## Materials and Methods

### Animals

108 SPF virgin female Wistar rats (aged 3–4 months, weight 200–220 g), obtained from the breeding facility IEP SAS Dobrá Voda, Slovakia, were used. The animals were housed under standard conditions. Food and tapwater were provided *ad libitum*. After 7 days of adaptation the females were mated with males (presence of spermatozoa in vaginal smear indicated day 0 of gestation)

### Drugs

STO was prepared by Štolc et al (1983) in the Institute of Experimental Pharmacology SAS, Bratislava, Slovakia. STO in the form of dipalmitate salt DP 1031 (m.w. 715.2, 99.5% purity) was dissolved in 0.5% methylcellulose (Methocel, MC 4000 cP, Fluka AG, Busch SG, Switzerland) at a constant dosage volume of 0.5 ml/100 g body weight. The dams were treated by oral gavage with STO in single doses of 5, 15 and 50 mg/kg/d from the 6th day of gestation up to weaning of pups – day 21 post partum. Controls received as vehicle 0.5% MC over the same period.

### Behavioural testing

The development of offspring of STO treated dams was followed up from birth up to postnatal day (pnd) 140. The neurobehavioural development was evaluated according to Adams (1986) and Hass et al (1994). We investigated **Somatic growth and maturation** (pnd 1–39) – body weight, unfolding of external ear, incisor eruption, development of fur, ear opening, eye opening, testes descended, vaginal opening.

**Neuromotor and reflex development** – righting reflex (pnd 5) – the rat's ability to turn over from supine position, negative geotaxis (pnd 8) – the rat's ability to turn 180° on a 25° incline placed head down, forelimb grip strength (pnd 13) – ability to hold on to a thin wire, performances on rotating rod (pnd 20).

**Sensory function** – startle reflex (pnd 15) – the presence or absence of sensorimotor reaction (jerks) to auditory stimulus expressed in percentages of pups.

On pnd 21 the pups were weaned and divided into male and female groups (10 males and females in each experimental group). Further variables of neurobehavioural development were evaluated in each gender separately.

**Levels of activity and emotional reactivity** – exploratory behaviour (motor and vertical activities) and defecation rate (number of boluses found after testing) in the open field (pnd 90). The motor (horizontal movement of the rat) and vertical (rearing, both forepaws lifted off the floor) activities were recorded by an electronic apparatus IMAC 48 (developed in the Department of Psychology, Philosophical Faculty, Comenius University, Bratislava, Slovakia), the walls of which contained photocells. The rat was placed in the centre of the experimental box sized 42 × 42 cm, constructed from transparent glass. Movement through a photocell beam sent a single pulse to the computer. All animals were tested in 6-min sessions once daily for 4 consecutive days in the same time interval between 08 00–12 00 a.m. For further details see Dubovický et al (1997).

### Learning and memory processes

*Interrupted habituation* (pnd 90) – decline of the intensity of motor activity during repeated exposure of the animal to open field during 4 consecutive days

*Uninterrupted habituation* (pnd 90) – decline of the intensity of motor activity during one 6-min session on the 1st day of testing in the open field

*Maze learning* (pnd 120) was performed in a double-Y maze. Water deprived animals had over 8 days every day 4 consecutive trials to find water. Time latency to find the water was recorded with a stopwatch.

*Aversive learning* (pnd 140) was conducted in a special box sized 40 × 30 × 30 cm with dark walls and with a bottom made of metal lattice. Unconditioned stimuli (electrical impulse coming from the lattice, 0.5 s, 65 V) and conditioned ones (light from the lamp placed above the box with a 60 W bulb) were used to create aversive (conditioned) learning. Animals were tested every day in 5 consecutive trials for a total of 4 days. The rate of expected reactions of the animals, i.e. jumping up to the cover of the box after the light had been switched on (aversive learning) was evaluated.

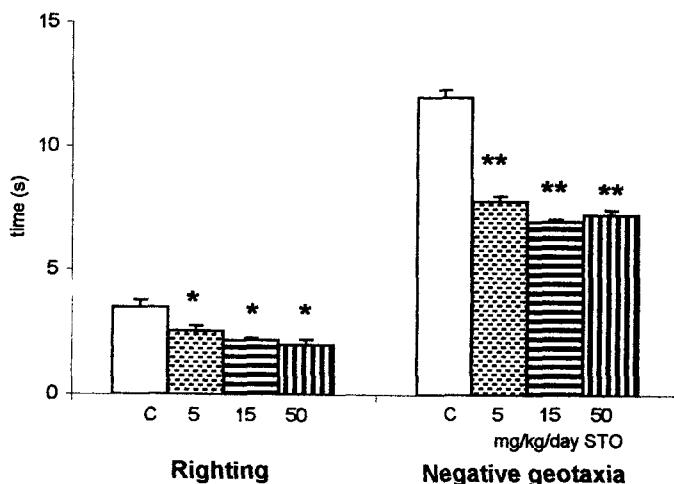
*Statistical evaluation* The data were analysed by means of ANOVA and are expressed as mean ± S E M. The exponential function  $Y(t) = Y_0 e^{-kt}$  ( $Y$  = amount of motor activity in individual sessions,  $k$  = individual rate of habituation,  $t$  = time of sessions) was used as a model of the habituation course of motor activity.

## Results

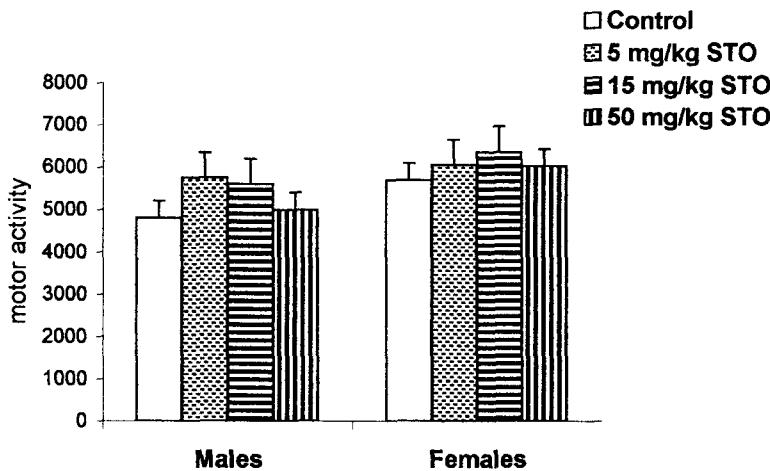
Oral administration of STO was well tolerated and no dam aborted or died. Neither did any of the pups die during the whole experiment. The individual variables of somatic growth and maturation in the pups of dams treated with STO were not significantly altered in comparison with controls (Table 1). Concerning neuromotor and reflex development, we found a statistically significant decrease in time latency of the righting reflex and of negative geotaxis in all STO groups (Fig. 1). No

**Table 1.** Effects of STO administration during pregnancy and lactation on somatic growth and maturation of rat pups. Presented data indicate age (postnatal days) of offspring at appearance of relevant developmental landmarks

	Dose (mg/kg/d)			
	Control	5	15	50
No of pups	100	65	97	100
Unfolding of external ear	2–3	2	2–3	2–3
Incisor eruption				
– lower	8	8	8–9	8–9
upper	10–11	9–12	10–12	9–12
Development of fur	10–11	9–12	10–12	11–12
Ear opening	12	12–13	12–13	11–13
Eye opening	14–15	13–15	14–15	13–16
Testes descent	23–32	22–32	23–32	23–33
Vaginal opening	32–38	31–38	31–39	30–39

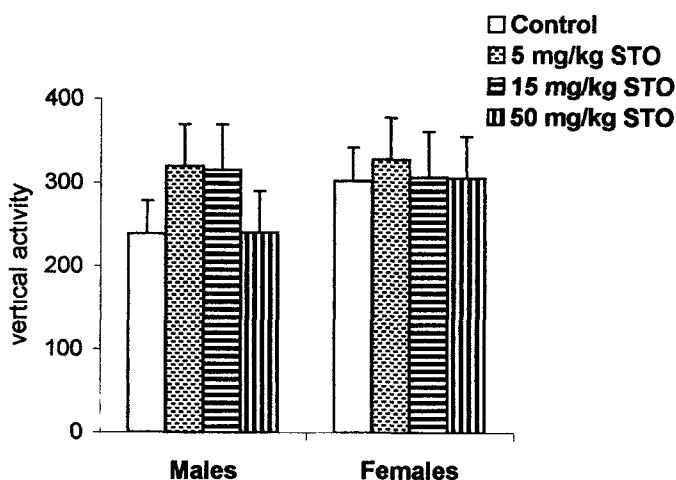


**Figure 1.** Effects of STO administration during pregnancy and lactation on righting reflex and negative geotaxia of pups. The height of columns represents the latency time for relevant event, vertical bars in columns denote S.E.M. (C – control). \*  $p < 0.02$ ; \*\*  $p < 0.01$  (significant difference compared to control).

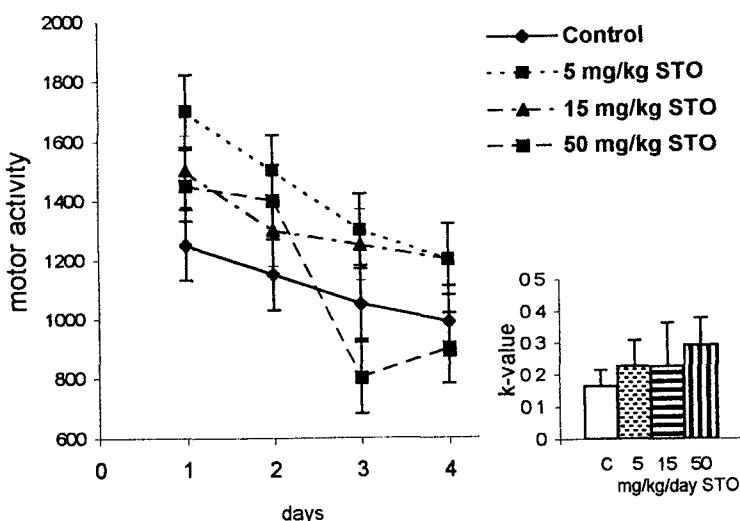


**Figure 2.** Effects of STO administration during pregnancy and lactation on the intensity of motor activity in offspring.

significant differences were found in the percentage of pups non-reacting to auditory stimulus (startle reflex) between control and STO-treated groups (data not shown).



**Figure 3.** Effects of STO administration during pregnancy and lactation on the intensity of vertical activity in offspring



**Figure 4.** Effects of STO administration during pregnancy and lactation on the course and individual rate of interrupted habituation (k-value) in male offspring (C - control)

As to open field testing, there were no significant changes either in the intensity of motor and vertical activities (Figs. 2, 3) or in emotional reactivity (data not shown) in males and females of the STO groups compared to controls.

Habituation of motor activity of offspring (interrupted as well as uninterrupted habituation in both genders) from the STO groups were not significantly altered compared to controls (Fig. 4) Similarly, no significant differences were found in aversive and maze learning of rat offspring of either gender of the STO groups compared to controls (data not shown)

## Discussion

Long-term administration of STO did not markedly influence the individual variables of neurobehavioral development of the rat offspring, with exception of their neuromotor development. The offspring exposed to STO via their mothers seem to be more skillful and mobile than controls. Acceleration of neuromotor development in the preweaning period was not accompanied by alterations in motor skills during weaning (rotarod) and adulthood (open field). After prenatal exposure to diphenhydramine, a classical H<sub>1</sub> receptor antagonist, a similar acceleration of neuromotor development was found in the rat offspring without long-term changes in adults (Chiavegatto et al 1997). On the contrary, perinatal treatment with glucocorticoids (dexamethasone) in rats resulted in preterm maturation of motor functions associated with deficit in motor performance and coordination in adults (Benešová and Pavlík 1989). A marked H<sub>1</sub>- and a weak H<sub>2</sub>-antihistaminic effect of STO was observed in respiratory smooth muscle and in the central and peripheral nervous system in the cat (Lukovič and Machová 1988). According to Altman and Sudarshan (1975), three peripheral systems may be involved in the regulation of postural adjustment, i.e. vestibular, exteroceptive and proprioceptive systems. In the development of these peripheral systems, the modulatory action of histamine can not be excluded. Exposure of the rats to STO during gestation and lactation improved both reflexes, suggesting an acceleration in the maturation of the systems controlling these behaviours in the offspring.

In conclusion, the results of our experiments showed that long-term administration of STO to dams during gestation and lactation had no adverse effects on the neurobehavioural development of their offspring.

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