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Serotonin involvement in the spontaneous alternation ability: a behavioral study in tryptophan-restricted rats

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Abstract

Spontaneous alternation (SA) is controlled by septal cholinergic terminals in the hippocampus. Serotoninergic terminals end on cholinergic nerve endings in the hippocampus, and their possible role in SA was investigated in rats fed with a tryptophan-deficient diet, from weaning to 60 days of age. A T-maze was used for the test. At the age of 40 days, an increase in SA occurred in the tryptophan deficient rats, although this effect disappeared by 60 days of age. A modulatory role of serotonin in the psychoneural control of SA is suggested, and it may be through presynaptic inhibition of hippocampal cholinergic terminals.

Keywords: Exploratory behavior; Spontaneous alternation; T-Maze; Serotonin; Tryptophan; Acetylcholine; Hippocampus

Discrimination among significant and non-significant environmental stimuli leads to the spontaneous alternation (SA) behavior, as an element of exploratory behavior. It consists of exploring the alternative arm of a T-maze, after the subject has selected the other one in a first exposure to the maze [20].

A close relationship between SA ability and hippocampal cholinergic activity is well documented [3,13, 15]. Cholinergic excitatory activity on hippocampal neurons [8,12] leads to an inhibition of the behavior; this consists of the suppression of a persistent and sometimes useless response. Thereafter, the alternate behavior takes place.

Cholinergic release is affected by presynaptic inhibitory serotoninergic terminals [17,24]. This interaction has been shown to affect several cognitive processes such as learning [11], long- and short-term memory [11], as well as spatial orientation [16]. Acute pharmacological studies have suggested that such neurochemical interaction is essential to the optimal expression of these psychoneural processes [18].

Serotonin (5-HT) has been strongly associated with the persistent behavior of obsessive-compulsive disorder [2,23], a neurotic condition in which alternation ability is limited [23]. However, the neurobehavioral meaning of serotoninergic activity alone in SA expression has not been studied. Therefore, chronic restriction of TRY (the precursor of 5-HT synthesis) in the diet is considered an adequate approach to investigate the role of 5-HT in the neural organization of SA and its long-term 5-HT-dependent impairments.

A TRY-deficient diet (0.15 g/23 g protein per 100 g diet) was provided ad libitum to 20 Sprague–Dawley female rats, and 20 control animals were pair-fed with conventional Chow (Table 1), from weaning (21 postnatal day) until they were 60 days of age. At 21, 40 and 60 days, animals were weighed; the behavioral test was applied when the rats were 40 and 60 days of age. Rats were kept at 25°C, with a light-dark cycle (12:12; 0700–1900 h) throughout the study. After 30 min of habituation

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Table 1
Composition of diets (g/100 g diet)

Components	Control	Experimental
Chow	98.0	–
Gelatine	–	27.0
Tryptophan	–	0.15
Vegetal oil	2.0	5.0
Vegetal fat	–	8.0
Glucose	–	19.0
Saccharose	–	20.0
Dextrin	–	7.0
Mineral mixture	–	2.0
Vitamin mixture	–	2.0
Non-nutritive fiber	–	10.0
% of protein	23.0	23.0
kcal/100 g	400.0	393.0

in a room illuminated by red light [4], at 0100 ± 2 h, 10 rats from each group were exposed to a novel T-maze, starting from the middle arm. After the animal had turned to either of both alternatives, it was withdrawn and isolated in a resting cage for 30 s; thereafter, the animal was placed in the starting arm once again. It was considered that SA had occurred only if the animal selected the alternative unvisited arm, in the second trial. Student's *t*-test and Fisher's exact probability tests were used to evaluate the significance of differences found between corresponding groups.

At both 40 and 60 days of age (Fig. 1) body weight gain in the experimental group of rats was significantly lower than that seen in the control group. On the other hand, SA ability of the TRY-restricted animals increased at the age of 40 days. There was no statistical difference in that parameter in 60-day-old rats (Fig. 2).

In female rats a higher activity of 5-HT synthesizing enzymes, greater storage capacity for 5-HT in brain serotoninergic neurons, and enhanced 5-HT-dependent behaviors are typical [5]. The latter parameter was evaluated in the present work, in order to explore the behavioral differences between the two groups studied.

Among other metabolic roles, TRY stimulates hepatic protein synthesis [14]. This metabolic process reaches its maximal peak before weaning, and TRY represents only 1.5% of the total amino acid content of total protein [14]; thus weight difference between control and experimental groups at 40 and 60 days of age were likely due to a generalized growth retardation, possibly due to a suppression of growth hormone (GH) release. In this respect, it is well known that TRY stimulates the secretion of GH, which is episodically secreted by 22 days of age [19].

On the other hand, since TRY is a precursor of 5-HT [6], and since brain 5-HT availability depends upon blood TRY levels [6,9,21], a direct relationship between TRY restriction-induced low brain 5-HT levels with the behavioral observations, is postulated. Furthermore, TRY restriction over 70% has been reported to have no effects on the concentration of other neurotransmitters [22]. In the current study, TRY was reduced by 50% in the experimental diet, with regard to control Chow. SA mainly depends upon hippocampal cholinergic activity [3,13,15]. The excitatory action of acetylcholine [8,12] leads to an inhibition of the behavioral effect [7,10]; thus, the animal discontinues a repetitive and sometimes useless psychomotor response. In a T-maze, SA is observed in terms of the choice of the non-visited arm, once the animal has visited the other arm at a first trial; this is mainly due to the lack of any significant stimulus or reinforcement. However, septal cholinergic terminals in the hippocampus are presynaptically inhibited by 5-HT-ergic raphe-projecting nerve endings [17,24]. Therefore, it has been postulated that 5-HT-ergic activity may play an important role in SA behavior.

There was a significant increase in SA ability at the age of 40 days. This is presumably due to a regulation of behavioral expression where postsynaptic inhibition on cholinergic terminals might be involved. In TRY-

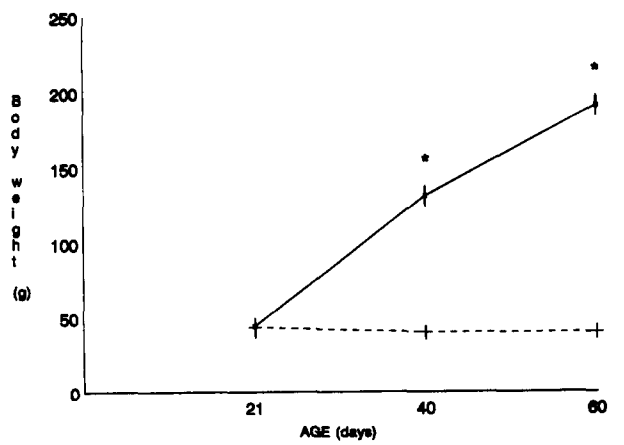

Fig. 1. Effect of a tryptophan-restricted diet on postnatal body weight gain. Continuous line, control group; dotted line, restricted animals. Values represent means ± SDM. *Significantly different from corresponding control group at $P < 0.0001$.

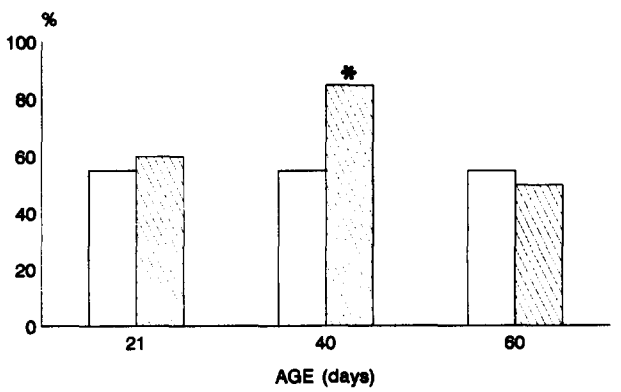

Fig. 2. Percentage of spontaneous alternation in a T-maze in tryptophan-restricted rats. Empty bars, control group; cross-hatched bars, restricted animals ($n = 20$ animals per group). *Significantly different from its control group at $P < 0.04$.

deficient animals, it is assumed that 5-HT production in the brain is reduced probably leading to a partial disinhibition of cholinergic terminals followed by an augmentation of excitatory control of the hippocampus with increased SA ability.

There was no significant difference in SA between both groups at the age of 60 days (Fig. 2). This may be due to a supersensitivity phenomenon including a reduction of 5-HT neurotransmission. It has been reported that infusion of TRY into TRY-restricted rats leads to compensatory changes either at 5-HT neuronal level or at 5-HT-ergic postsynaptic receptors [1]. The latter may be involved in the control of SA expression. Further neurochemical studies are required to elucidate which receptors are involved in these processes.

In conclusion, we postulate that 5-HT is involved in the SA behavior and that the experimental model used is an adequate approach to study the chronic plastic events related to the possible participation of 5-HT-ergic neurotransmission in some neuropsychopathological disorders, such as obsessive-compulsive neurosis [2,23].

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