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Research report

Postnatal exposure to predator odor (TMT) enhances spatial learning in mice adulthood

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ABSTRACT

Adult behavioral and physiological responses are partly dependent on neonatal experiences. In several animal species, enriched/aprovished environments and stressful/appeasing events are crucial in the setting of adaptative behaviors. However, little is known about the effects of postnatal exposure to predator odor (as unconditioned fear-related stimulus) on spatial learning at adulthood. Thus, the aim of the present study was to investigate the effects of a postnatal exposure to 2,4,5-trimethylthiazoline (TMT, as a predator odor) on radial arm maze (RAM), Tolman maze (TM) and Morris water maze (MWM) in mice at adulthood. The results showed that a *TMT group* constituted by mice exposed postnatally during 3 weeks to TMT presented significantly better spatial learning achievements in adulthood compared to a *water group,* postnatally exposed to water only, as well as compared to a *butanol group* (butanol used as an odor without ecological significance) exposed postnatally to butanol during 3 weeks.

1. Introduction

Environmental stimuli in early life may result in behavioral and physiological changes at adulthood. Indeed, neonatal experiences are imprinted on the brain by affecting its development and consequently induce long-term effects including adult learning and memory functions. In rodents, there are many studies describing the effects of a novel or unfamiliar environment, adverse experiences such as "handling" or stressors (e.g. noise, electric shock or proximity to an unfamiliar adult male) during early postnatal periods on later adult life [42,32,38,2,16,34,35].

Historically, learning is referred as a more or less permanent change in behaviors that occur as a result of practice [18]. Learning and memory are thought to be a continuous process in which learning modifies subsequent behaviors while memory is the ability to remember past experiences. The major brain structure involved in this process is the hippocampus given that the acquisition of motor skills and habits is also mediated by neostriatum and cerebellum [33] and that amygdala has been shown to be involved in memory consolidation [4]. From an adaptative point of view, spatial learning is crucial for many animal species including most of mammals, e.g. territory identification, food search, detection of congeners or predators.

Concerning spatial learning and memory in rodents, it has been shown that chronic postnatal stress can influence abilities in adulthood [5] in response to the same or another stressor [37,9]. Moreover, it must also be considered that neonatal experiences – such as maternal deprivation – can affect spatial learning and memory in adulthood without any new events preceding acquisition [26,46] as well as in the case of gestational stress [24]. In contrast, neonatal enriched environments improves memory and prevents stress-induced learning and memory impairments [8].

In rodents, olfactory cues are crucial in adaptative behaviors especially towards predator odor as unconditioned fear stimulus. In the case of fear-related studies, the synthetic predator odor 2,4,5-trimethylthiazoline (TMT) a component of natural fox feces, is frequently used to induce behavioral and physiological responses in a threatening situation [11,3,13]. Surprisingly, little is known about the effects of postnatal TMT exposure at adulthood. In a recent study [14], the effects of a postnatal exposure to synthetic predator odor (TMT) in mice pups were investigated on later adult fear-related behaviors and corticosterone levels in response to this specific stimulus. The results showed that adult mice exposed as neonates to TMT presented less fear-related behaviors than controls while no differences occurred in the corticosterone levels between both groups. These findings suggested that the mechanism implied could be a learning process allowing to adapt the fear-related behavioral responses.

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In order to explore further the consequences of posnatal exposure to TMT, the aim of the present study was to investigate the effects of a 3 weeks postnatal exposure to synthetic predator odor TMT on subsequent adult spatial learning in mice, without any new events preceding acquisition, especially stressful situations, except food deprivation. Behavioral parameters were recorded in three different complementary mazes, i.e. radial arm maze (RAM), Tolman maze (TM) and Morris water maze (MWM). RAM allows to investigate "working memory" and "spatial reference memory" during training and retention sessions. Furthermore, chronic stress and hippocampal lesions are known to impaired acquisition on the RAM, in spatial reference as well as working memory errors [29,27]. The TM permits to investigate the use of spatial memory when changes appear in a well known space and to evaluate the efficiency of strategies by the account of success and errors. The MWM is known to be highly sensitive in the assessment of spatial learning and memory including damages to the hippocampus. It permits to have a learning parameter expressed in time corresponding to latency to find the platform. Moreover, the MWM does not require previous preparation (such as food deprivation) and presents the advantage that water eliminates the possibilities that animals use aromatic cues in the escape search, one potential confounding factor in the dry-land mazes.

Results of the mice group postnatally exposed to TMT (named *TMT group*) were compared to results of a mice group postnatally exposed to water only (named *water group*) and a mice group exposed to butanol as an odor without ecological significance (named *butanol group*).

2. Methods

Animals: The animals were offspring of female OF-1 mice. Pregnant females were purchased from Charles River (France). When females delivered, they were housed with their own pups in home polycarbonate cages (type E: depth, 40.5 cm; width, 25.5 cm; height, 19.7 cm; floor area, 1,032.75 cm^2) with open stainless steel wire lids (Charles River, France). The animals were maintained in room at constant temperature (22 ± 1 °C), constant humidity (45–55%) and at constant luminosity (350 ± 30 Lux) under a cycle with light phase of 12 h from 8 p.m to 8 a.m. Animals had free access to food pellets and water. The study was carried out in accordance with the "Guide for the care & Use of Laboratory Animals" (National Institute of Health, USA, 1985).

Postnatal Exposures: At postnatal day 1, three groups of females with their pups were constituted and exposed (30 min per day, 5 days per week during 3 weeks) in different conditions: a group exposed to 1% TMT (named TMT group), a group exposed to distilled water (named water group) and a group exposed to 10% butanol (named butanol group). During the exposure periods, mice were placed in an inhalation chamber (long: 80 cm; wide: 42 cm; deep: 41 cm; volume: 0.13 m^3). Odorants were placed on a cotton in a open glass in the exposure chamber: for the *TMT group*, 150 μl of 1% TMT ($C_6H_{11}NS$, PheroTech, Britannic Colombia, Canada) diluted with an agitator in distilled water, for the *water group* 150 μl of distilled water, and for the *butanol group* 150 μl of 10% butanol. Two silicone hoses (1 m length, 1 cm diameter) placed into two sides of the exposure chambers allowed passive ventilation during exposure periods. When mice were 12 weeks old, three groups of 10 females (in order to avoid a possible gender effect) were constituted for each exposure condition, i.e. TMT, water and butanol. For each behavioral test (RAM, TM, MWM), one group of 10 mice of each exposure condition was used. A group of 10 female mice was constituted from 3 or 4 litters. Moreover, females were housed 10 per cage in order to induce a homogeneous hormonal state in the group, i.e. anestrus. All animals ran through all tests in a randomized order.

2.1. Behavioral tests

Radial arm maze (RAM) [28] adapted for mice (6 cm wide and 35 cm long) consisted of 8 arms with a central platform (18 cm in diameter). In a preliminary session, animals explored freely the maze before the tests without any food pellets in two trials (5 min each). Behavioral tests were conducted in two sessions named training session and retention session. The training session consisted of one trial (5 min) a day three days on a week (Monday–Wednesday–Friday). In the same way, the retention session was conducted the following week. For both training and retention sessions mice were food deprived during the 12 h before each trial. A pellet of food was alternately placed at the end of four arms. Animal who has been food deprived was placed in the center and was allowed to collect food pellets from each baited arm (sampling without replacement) located by a visual cue. The optimal strategy for obtaining all the food in the least amount of time was to visit each arm only once during a trial. The number of visits to empty arms (unbaited arms) was calculated as errors made [41]. Re-entry into a previously baited arm (now empty) was defined as "a working memory" error. Entries into never-baited arms were counted as "spatial reference memory" errors.

Tolman maze (TM) consisted of three ways (6 cm wide) from departure to arrival with direct (50 cm), short (80 cm) and long route (110 cm). In a preliminary session, animals explored freely the maze before the test session in two trials (3 min each) with a rest period of 30 min. In the first trial, food was not available. In the second trial food was placed at the arrival and every time the animal arrived to the food, it was immediately replaced to the departure. The test sessions consisted of two trials corresponding to two door positions, one closing the direct route only (i.e. the good choice was the short route and the errors were counted when the mouse entered in the direct and the long routes), another closing the direct and the short route (i.e. the good choice was the long route and the errors were counted when the mouse entried in the direct and the short routes). The order for both trials was randomized between animals. For the test session, mice were food deprived during the 12 h before each trial. Every time the animal arrived to the food, it was immediately replaced to the departure. The measured parameters were the number of success (i.e. the animal arrived to the food), the number of entries in the right route (only one possibility) and the number of entries in the wrong routes (two possibilities).

MWM consisted of a circular pool in which mice were trained to escape from water by swimming to a slightly hidden platform (2 cm) below the water level. The pool was divided into four quadrants named NE, NW, SE and SW. The platform was placed in the middle of the SW quadrant and remained at the same position during the whole experiment. Water temperature was maintained at 24 °C.

The spatial acquisition consisted of 16 trials: 4 trials per day (with an inter-trial interval of 20 min) during 4 days. Mice were released randomly from the four compass locations and allowed to swim and search the platform for 120 s. If mice did not locate the platform after 2 min, animals were manually placed on the platform and allowed to remain on it for 30 s. The time required to discover the escape platform was recorded for each trial.

2.2. Statistical analyses

Data were statistically evaluated with Statview 5.0 software using ANOVAs and Scheffé post hoc tests. Data were expressed as means ± standard errors. The significant level was set at 0.05. The non-significant results were noted as *ns*.

3. Results

3.1. Radial maze

Working memory errors: Results of working memory errors are reported in Fig. 1. The ANOVA showed a group effect ($F_{2,29} = 13.89$, $p < 0.0001$), and a session effect ($F_{1,29} = 66.31$, $p < 0.0001$), i.e. logically mice made less working memory errors during the retention session than during the training session. No significant interaction was observed between the variables group and session ($F_{2,29} = 0.53$, *ns*). Post hoc Sheffé tests showed that the *TMT group* made significantly less working memory errors in the training session than the *water group* ($F = 2.77$, $p < 0.05$) and than the *butanol group* ($F = 4.75$, $p < 0.05$) while no difference occurred between the *water group* and the *butanol group* ($F = 0.68$, ns). In the same way, post hoc Sheffé tests

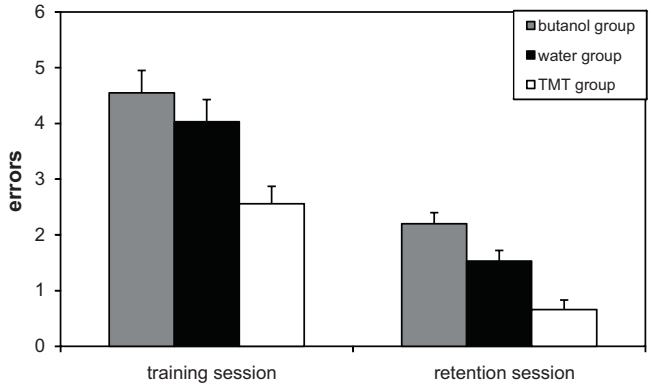

Fig. 1. Working memory errors in a RAM during training and retention sessions for three mice groups postnally exposed to butanol, water and TNT.