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Research Report

Modest environmental enrichment: Effect on a radial maze validation and well being of rats

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Abstract

Our 8-arm radial maze test was validated to demonstrate memory deficits in rats treated with the muscarinic antagonist scopolamine hydro bromide (SHB, 0.1 mg/kg, i.p.). To improve quality of life, we enriched the environment of single housing rats. Enrichment procedures were chosen to increase the animals' well being without disturbing a lot the results of behavioural tests. It is modest, consisting of a plastic tube and corn chips. Enriched environment (EE) and Non-enriched Environment (NE) animals' performances were compared during the 8-arms radial maze validation. Enrichment procedures were chosen to increase the animals' well being without disturbing the results of behavioural tests. The impact of our enrichment conditions was then evaluated on the general behaviour of rats, weight evolution and results of a plus maze anxiety test. Results showed a deficit and a delay in learning for SHB-treated animals, and a general time-dependent learning effect, validating our test. No effect of enrichment on negative control animals was observed. For SHB-treated animals, enrichment increased performances during learning task and accentuated the deficits in test task. Exploratory behaviour of enriched animals seemed to be increased. A general amelioration of well being for EE animals was found (stable weight). We conclude that our enrichment allows increasing exploratory behaviour not modifying radial maze sensitivity using a simple modification of our protocol (limitation to 16 visits/trial). We decided to generalise this enrichment to all our studies, given its simplicity and obtained benefits.

Theme: Neural basis of behavior
Topic: Motivation and emotion

Keywords: Enriched environment; Single housing; Well-being; Radial maze; Plus maze; Rat; Scopolamine

1. Introduction

The 8-arms radial maze is specific of learning and memories, using different protocols [31,45]. An eight baited arms protocol was followed to test working memory. To validate our test, reference drugs were used: scopolamine hydro bromide (SHB) and scopolamine methyl bromide (SMB) [13,17]. SHB is a specific cholinergic antagonist, able to cross the blood–brain barrier (BBB), involving effects on peripheral nervous system (PNS) and central nervous system (CNS). SMB is also a specific cholinergic antagonist, but the methyl group prevents BBB crossing. Centrally, acetylcholine (ACh) plays a specific role in learning and memory processes [4]. Central antagonist effects of scopolamine involve impairing working memory in radial maze task by increasing the number of working memory errors (visits of already visited arm) and delay to enter in arm and decreasing motivation [5,24,38,47,55]. In periphery, ACh is the neurotransmitter of parasympathetic system, reducing energetic consumption. Peripheral effects obtained [41] affect task results without impairing learning and memory [23,54]. With the SMB, only peripheral effects are obtained. This treatment was used as the SHB control group to determine the possible observed effects resulting from a non-specific impairment of learning or memory.

To control the weight during radial maze task (food motivation), animals were singly housed. In accordance to

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ethics and legislation [11,12,28,32], alleviating stress from single housing and deprivation was imperative. To improve well-being and reduce stress, we decided to enrich the animal's environment.

Housing environment has an impact on biological mechanisms underlying animal behaviour, and slight changes during an experiment can alter responses [1,25]. Laboratory animals are deprived of some natural behaviours and that induces suffering and a decrease of well being [9,26,51]. Environmental enrichment (EE) allows welfare improvement, and stress level decreases by giving more behavioural possibilities. Animals consider it as a recompense [2,42,50]. Several studies looked at the effects of EE at physiological and behavioural levels [10,39,52]. Enrichment increases brain weight, cortex development, which has beneficial effect on performance of rats in behavioural tasks. Moreover, EE could reduce effects of various affections (stress, non-handling, brain lesion, ischemia, gliosis) and could be used as treatment for them [19,29,33,37,46]. The term "enrichment" covers social and inanimate stimulation [40], but these two kinds of enrichment have dissociated effects [43,44,58]. Social stimulation consists in putting animals together. Indeed, single housing affects physiological parameters by increasing stress level, increasing locomotion activity and decreasing exploration [14,22]. In learning behavioural tasks, isolated animals have worse performances than enriched and socialised ones [8,15,20]. Isolation effects seem to be strain dependent [52] and can be reduced by adding inanimate enrichment in the cage [3]. That consists in putting objects or enlarging cage dimensions and increasing the complexity of the environment and of behavioural possibilities. Rats prefer large cages and objects to chew and to nest [6,34,35]. Behavioural benefice of enrichment has been observed in an open field task: quality of exploration is increased, whereas locomotion behaviour decreases [7,18,53]. These effects depend on the enrichment complexity but not on duration of enrichment [48,58]. Besides, only few elements are needed to obtain results [56].

To compare our results with those of previously published studies, EE had not modified too much the radial maze task results. The enrichment was chosen to be modest and to not complicate the animal facilities organisation. We test its effect during the validation of the radial maze task. To test the impact of our enrichment on welfare, a plus maze test was then performed. This behavioural test is widely used to measure anxiety-like reactions and exploratory behaviour in various pharmacological studies with rodents [16,27,36]. Furthermore, as the radial maze, the plus maze is constituted of unclosed elevated arms. It was chosen in order to allow an easier comparison between results of two tests. In these conditions, animals were not deprived. Their weight was measured daily as a well being index, and general attitude (coat aspect, facility of contention, behaviour in the cage) was observed.

The first aim of the present study was to validate in our laboratory a working memory protocol in the radial maze using drugs. The second aim was to compare enriched animal's performances to the controls during the validation of the radial maze task and to measure the well-being increase due to enrichment.

2. Materials and methods

2.1. Radial maze experiment

2.1.1. Animals

Thirty-two male Sprague–Dawley rats (OFA Iffa Credo, France), weighing 160–180 g on arrival in the laboratory (6 to 7 weeks old), were used. Environmental ambient conditions were controlled: ambient temperature 22 ± 1 °C; hygrometry 50% ± 2%; pressure + 2 mm Hg; 12 h light/dark cycle. Experimental design showing different habituation phases is detailed in Fig. 1. Animals were first housed 4 per cage with food and water ad libitum and left 5 days without any manipulation to be accustomed to animal facilities. Later on, study experimenters handled them 10 min a day during 8 days. After 3 days of experimenters' habituation, rats were randomised and placed 1 per cage with water ad libitum but controlled food quantity. To reinforce their motivation, food was reduced to obtain a weight decrease of 10 to 15%. Animals were weighted daily at the same hour, and food quantity was adapted according to the theoretical weight curve (IFFA CREDO documents). Pellets (20 mg; Phymep SARL, France) were given as reinforcement food.

2.1.2. Experimental groups and treatment

2.1.2.1. Housing conditions. Half the animals (n = 16) were housed in standard plastic laboratory cages, in Non-enriched environment (NE) condition (Type III H, 425 × 266 × 185 mm; sawdust + water + food) and the other half in enriched cages, EE condition. This enrichment consisted in adding corn chips (2 cm thickness) and a translucent plastic tube (12 cm length; 7 cm diameter).

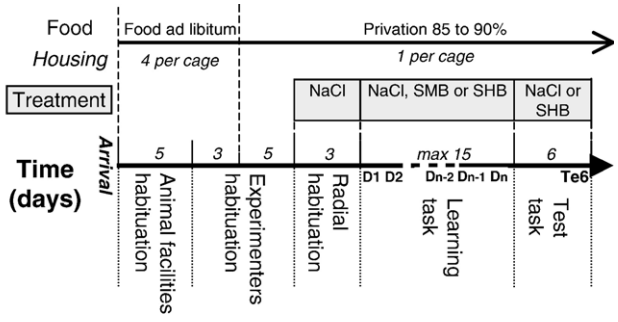

Fig. 1. Experimental design of Experiment 1.