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Research report

Evaluation of an $A\beta_{1-40}$-induced cognitive deficit in rat using a reward-directed instrumental learning task

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HIGHLIGHTS

- Our laboratory is the first to utilize a reward-directed instrumental learning procedure in a dementia study.
- $A\beta_{1-40}$ injection affects the acquisition of the lever-pressing response rather than its maintenance.
- $A\beta_{1-40}$ injection impairs the ability to adjust behavior to adapt to action–outcome contingency changes.
- $A\beta_{1-40}$ injection impairs the ability to make a stimulus–response association from a two-color visual stimulus.
- This series of instrumental learning tasks has the potential to be applied in dementia research as a new cognitive behavior testing method.

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia. It is a progressive neurodegenerative disorder that leads to gradual loss of cognitive and functional abilities, and development of behavioral disturbances. Previous studies using $A\beta_{1-40}$ microinjection in animal models focused on cognitive deficits in spatial learning and avoidance conditioning. However, no attempt has been made to determine the sensitivity of an $A\beta_{1-40}$-manipulated animal model in tasks involving reward-directed instrumental learning (RDIL). Thus, the present study was designed to investigate the effects of intra hippocampal microinjection of $A\beta_{1-40}$ on the acquisition and maintenance of a basic instrumental response (lever-pressing), then on the goal directed (higher response ratio) and habit (visual signal discrimination and extinction) learning, as well as on neurotransmitter changes which could potentially alter the regulatory processes involved in instrumental learning. Our present findings demonstrated that the focal hippocampal microinjection of $A\beta_{1-40}$ rendered rats unable to process new cue/contextual information in the formation of causal relation, rather than affecting the operant action itself. Although the injected $A\beta_{1-40}$ did not directly influence performance, it did prevent the information from being translated into action. Moreover, the neurotransmitter results indicated that multiple neural signaling might be involved in the regulation of RDIL in the $A\beta_{1-40}$ injection model. In conclusion, results suggested that our series of instrumental learning tasks may have potential in dementia research as a novel method for testing cognitive behavior.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly and has rapidly emerged as a major public health issue throughout the world [1]. It is a progressive neurodegenerative disorder that leads to a gradual loss of cognitive and functional abilities, and the development of behavioral disturbances [2]. Among regions of the AD brain, the hippocampus has one of the highest concentrations of amyloid-containing senile plaques [3]. An in vivo hippocampal microinjection of amyloid-beta peptides (Aβ) in rodents induced neurocytic denaturation and apoptosis, as well as cognitive deficits [4–7]. This type of manipulation caused impairments on spatial and non-spatial tasks which were considered to reflect damage to the functional integrity of the

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hippocampus [8]. This highly used laboratory model has contributed enormously to AD research over the years [9]. The principal behavioral tasks described in recent articles have been maze- [10–14] and avoidance- [12,15–17] based. Evaluation of spatial memory and avoidance conditioning has been the theme of most of these studies. In addition, punitive stimuli were commonly applied. From one perspective, the tasks employed above not only gave an incomplete description of the fundamental behavioral alterations in dementia but also caused harm to the subjects. Moreover, although some tasks using rewards were conducted, they were still aimed at assessing spatial cue learning [3,18,19]. Therefore, other aspects of cognitive deficits induced by Aβ are worth exploring to gain a better understanding of the disease.

Instrumental conditioning, which is also called operant conditioning, provides a very accurate model of goal-directed action. It is a form of associative learning through which an animal learns from the consequences of its behavior [20]. Result using a transgenic AD mouse model showed an age-dependent deficit in acquiring a positive appetite-driven Pavlovian context-outcome, but revealed no instrumental or cue-outcome associations [21]. Since, no attempt has been made as yet to determine how an animal model administrated $A\beta_{1-40}$ would response to a reward-directed instrumental learning (RDIL), we focused on this area in our search of the literature. Reward-guided conditioning is controlled by two memory systems: a goal-directed process and a stimulus–response habit mechanism that involves two forms of learning. The first consists of establishing incentive by introduction to the reward, whereas the second consists of making an association between the response and receiving the reward [22]. According to the associative theory, instrumental learning is mediated by cues (stimulus) that predict the reward (outcome) and goal-directed behavior is learned (response/action) to gain access to the reward. The capacity for instrumental conditioning depends critically on the ability to encode a causal relationship between the action and the outcome. In addition, to the pleasure aspects of natural reward (e.g. sucrose, food, etc.), the learning process itself would be more pleasant [23] and would even have the advantage of promoting neural circuit remodeling in the brain [24].

Substantial evidence from lesion study has delineated the roles of specific brain regions in behavior regulation during instrumental cognitive tasks. In these types of studies, researchers induce lesions in specific brain regions of animal models and subsequently test for operant behavior alterations. If the behavior is compromised, it suggests that the destroyed tissue is part of a brain region that is important to the normal expression of operant behavior. Although lesion studies can elucidate the neural basis of a behavior by disrupting function in a specific brain region, the lesion may lead to permanent tissue damage which is far from mimicking the pathological process in dementia. However, $A\beta_{1-40}$ only impairs neural transmission and does not damage the structure of the hippocampus [14,25]. Therefore, we speculated that the $A\beta_{1-40}$ model may share some aspects in common with the lesion study model but would have distinguishing influence on RDIL.

Consequently, the aim of the present study was to evaluate $A\beta_{1-40}$-induced cognitive deficits in RDIL. We first investigated the effects of pre-training and post-training intra hippocampal microinjection of $A\beta_{1-40}$ on the acquisition and maintenance of instrumental response (Experiment 1). Secondly, the sensitivity to changes in the action–outcome contingency degradation and the identification ability of different stimuli–response associations were assessed in the post-training surgically treated rats (Experiment 2). After completing all the behavioral procedures, the rats were sacrificed and each hippocampus was examined for neurotransmitter alterations.

2. Methods

2.1. Animals

Eighty-one male Wistar rats (Vital river, Beijing, China), 10 weeks old, weighing 300–320 g at the beginning of the experiments, were housed 4 to a cage with lights on from 7:00 h to 19:00 h. The rats were maintained at 85% of an adjusted ad libitum body weight throughout the duration of the study by restricting food to approximately 16 g per day. Once training began, they were fed each day after the training sessions, and had free access to water while in their own cages. All rats, regardless of group, received the same handling and feeding during this phase of the experiment.

All animal handling procedures were performed in accordance with the "Principles of Laboratory Animal Care" (NIH publication No. 86-23, revised in 1996) and P.R. China legislation for the use and care of laboratory animals. All efforts were made to minimize animal suffering during experiments. The protocols were approved by the committee for the Care and Use of Laboratory Animals of IMPLAD, CAMS & PUMC, China.

2.2. Apparatus

Behavioral testing was conducted in four operant chambers (Xin Hai Hua Yi Instrument Co., Beijing China) fitted with a dipper magazine and a retractable lever (4 cm wide, positioned 10 cm from the side walls, 2.5 cm to the magazine and 5 cm from the grid floor). Three-color LED signal lights were located 5 cm above the lever. The chambers could be illuminated by a LED house light located on the ceiling. An infrared beam emission and acceptance device was fixed on the side wall of the magazine to record the nose poke activity of the subjects. Ventilation and a masking noise were provided by an exhaust fan. The operant chambers were housed in sound-attenuated rooms.

2.3. Drug preparation

The drug used was amyloid β protein fragment 1–40 ($A\beta_{1-40}$) (Sigma–Aldrich, USA) which was dissolved in sterile double-distilled water at a concentration of 5 μg/μl and incubated at 37 °C for 7 days prior to use.

2.4. Surgical procedures

Rats were assigned to surgery groups in a quasirandom manner. Initial random group assignments were adjusted using baseline magazine training performance to control for a response bias. Animals were divided into three groups for use in Experiments 1A, 1B and 2 (see Fig. 1). These groups consisted of $A\beta_{1-40}$-injected ($n = 9$), sham-injected ($n = 9$) and non-operated control ($n = 9$) rats respectively. The surgically manipulated rats received either a sham or $A\beta_{1-40}$ injection into the dorsal hippocampus using the procedures described below.

Rats were anesthetized with 10% chloral hydrate diluted in physiological saline (3.5 ml/kg, IP) and placed into a stereotaxic apparatus (Benchmark, USA) with head held horizontally. A midline incision was then made into the scalp and the scalp was retracted. Small holes were then drilled into the skull above the injection sites using a dental burr. The stereotaxic coordinates to conduct a bilateral microinjection in the CA1 region of the hippocampus (anterior-posterior (AP) = −3.3 mm, medial-lateral (ML) = ±2.0 mm from the bregma and dorsal-ventral (DV) = 3.0 mm from cerebral dura mater) were standardized from the stereotaxic atlas of Paxinos and Watson [26]. A flatted-tipped Hamilton syringe lowered into the bilateral hippocampus and 5 μl of $A\beta_{1-40}$ were delivered at a rate of 1 μl/min. Following the injection, the needle was kept in place for 5 min prior to its slow extraction. Rats of the sham group were infused with the vehicle only. After surgery, animals were placed in heated chambers in a darkened room and allowed to recover with free access to food and water. The experiment was continued after 10 days of recovery as follows.

2.5. Behavioral task

2.5.1. Magazine training

When subjects were at 85% of their ad libitum weight, all of them were habituated to the operant box over three consecutive 20 min sessions in which the reward (approximately 0.2 ml) was delivered daily in diminishing amounts (30, 25 and 20 drops) on a random time (RT) (40 s, 48 s and 60 s) schedule. Throughout the experiment, the lever was retracted. Each session began with the onset of the house light and terminated with its offset after 20 min. During the magazine process, rats in the operant chamber were only exposed to a blue cue light and white noise. The blue cue light was simultaneously illuminated with the appearance of the reward which was an 8% (*m*/*v*) solution of sucrose in distilled water that was prepared daily before each session.

2.5.2. Experiment 1

A total of 54 rats was used to assess the effect of pre-training and post-training intra hippocampal microinjection of $A\beta_{1-40}$ on the acquisition and maintenance of the lever pressing response.