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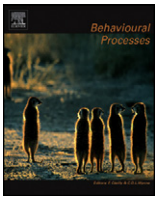

Recognition memory in tree shrew (*Tupaia belangeri*) after repeated familiarization sessions

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ABSTRACT

Recognition memories are formed during perceptual experience and allow subsequent recognition of previously encountered objects as well as their distinction from novel objects. As a consequence, novel objects are generally explored longer than familiar objects by many species. This novelty preference has been documented in rodents using the novel object recognition (NOR) test, as well is in primates including humans using preferential looking time paradigms. Here, we examine novelty preference using the NOR task in tree shrew, a small animal species that is considered to be an intermediary between rodents and primates. Our paradigm consisted of three phases: arena familiarization, object familiarization sessions with two identical objects in the arena and finally a test session following a 24-h retention period with a familiar and a novel object in the arena. We employed two different object familiarization durations: one and three sessions on consecutive days. After three object familiarization sessions, tree shrews exhibited robust preference for novel objects on the test day. This was accompanied by significant reduction in familiar object exploration time, occurring largely between the first and second day of object familiarization. By contrast, tree shrews did not show a significant preference for the novel object after a one-session object familiarization. Nonetheless, they spent significantly less time exploring the familiar object on the test day compared to the object familiarization day, indicating that they did maintain a memory trace for the familiar object. Our study revealed different time courses for familiar object habituation and emergence of novelty preference, suggesting that novelty preference is dependent on well-consolidated memory of the competing familiar object. Taken together, our results demonstrate robust novelty preference of tree shrews, in general similarity to previous findings in rodents and primates.

1. Introduction

Tree shrews (*Tupaia belangeri*) are day active mammals from Southeast Asia and are considered as intermediates between rodents and primates (Fuchs and Flügge, 2002; Martin, 1968; Petry et al., 1984). Genetic information is available, since the tree shrew was selected as one of 25 mammals for low coverage sequencing by the Broad Institute (http://www.broadinstitute.org/mammals). They are small slender mammals roughly the size of a rat with a long bushy tail, and exhibit agile and quick movements in arboreal environments (Lu and Petry, 2003). The small size of the animal combined with its close proximity to primates from an evolutionary point of view, uniquely places the tree shrew as an invaluable candidate for many behavioral tests. Nonetheless, the behavioral studies done with tree shrews have been rather limited when compared to more standard animal models such as the rat or non-human primate. One line of work has established tree shrews as a model animal for social stress (Fuchs, 2005; Fuchs and Flügge, 2002, 2003; Kozicz et al., 2008; Zambello et al., 2010). Other aspects of tree shrew behavior such as learning set formation (Ohta et al., 1985), visuo-spatial learning (Ohl et al., 1998; Ohl and Fuchs, 1999) and transitive inference (Takahashi et al., 2008) have also been investigated. In general, these studies show that tree shrews have competent cognitive abilities. For instance, all participating tree shrews were able to learn an inference task, whereas only a subset of rats achieved criterion performance on the same task (Takahashi et al., 2008). In addition, there has been some work on visually based behaviors (Callahan and Petry, 2000; Lu and Petry, 2003; Petry et al., 1984; Raczkowski et al., 1976), since tree shrews–unlike rodents – have a well developed visual system and can be readily trained to perform various visually based tasks. Given this well-developed and highly differentiated visual system, our aim was to extend the repertoire of tree shrew visual behavioral paradigms by examining their behavior in an object recognition memory test.

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Since its introduction (Ennaceur and Delacour, 1988), the novel object recognition (NOR) test has gained popularity as a recognition memory test in rodents. It consists of an arena familiarization, an object (usually a pair of identical objects) familiarization and, after a certain interval, a test phase. The NOR test has been used to investigate the influence of a broad range of manipulations on memory (Aubele et al., 2008; Fahey et al., 2008; Jurdak and Kanarek, 2009; Lockrow et al., 2010; Reger et al., 2009; Weible et al., 2009). This simple test is based on the premise that rodents inherently tend to preferentially explore a novel object in the presence of an already familiar object. This novelty preference necessitates an intact recognition memory of the previously seen familiar object. The NOR test has several advantages over other memory tests including the absence of any externally applied reinforcement or rule, making it particularly suitable for assessing recognition memory. According to the interval between object familiarization and test phases, typically spanning from one minute to over 24 h, the task is suited for examination of both short term memory (STM, 1–90 min interval) and long term memory (LTM, usually 24 h interval). Generally, rodents do not show significant novelty preference in the long term (24 h) memory test in the NOR task (Ennaceur and Delacour, 1988; Okuda et al., 2004; Roncarati et al., 2009; Tellez et al., 2010), with some notable exceptions (Botton et al., 2010; Reger et al., 2009). The possible reasons for largely absent LTM in rodents in the NOR paradigm have not been discussed extensively in previous literature.

The weak novelty preference observed after 24 h in rodents is thought to be due to a failure to maintain a memory of the familiar object over this period. This poor LTM provides an opportunity for testing memory enhancers (Okuda et al., 2004; Wong et al., 2010; Roncarati et al., 2009). By contrast, rodents do not provide a good model for assessing detrimental effects on LTM. Nevertheless, to date, almost all NOR tests have been done in rodents and this test is increasingly used in rodent animal models of various diseases including Alzheimer's Disease (Donkin et al., 2010; Greco et al., 2010; Hillen et al., 2010; Lu et al., 2009; Mouri et al., 2007; Nishida et al., 2006; Taglialatela et al., 2009), schizophrenia (Hauser et al., 2009; McLean et al., 2009; Neill et al., 2010; Ozawa et al., 2006; Powell et al., 2007; Vigano et al., 2009), and Down's syndrome (Lockrow et al., 2010). The absence of any external reinforcement in the NOR test is particularly important as it makes it more comparable to memory tests in human, which are normally conducted without the use of positive or negative reinforcers such as food and electric shock (Ennaceur and Delacour, 1988). Additionally, validating the NOR task in an animal model that is phylogenetically closer to humans could strengthen the extrapolation of animal studies to our understanding of human cognitive functions, as well as facilitate translational medicine in memory-related complications.

Here, we used the NOR task in tree shrew and showed robust novelty preference following three, but not one day of object familiarization. In addition, we found that exploration time for familiar objects was already reduced following a single object familiarization session. Our study provides novel insights into the dynamics of recognition memory formation in the tree shrew.

2. Materials and methods

2.1. Animals

Adult male and female tree shrews were housed in large cages that were connected through a tube to a nesting box. A total of 13 animals (4 males) aged 1–5 years old were used in the experiments. They were kept in a temperature controlled room and maintained on a standard 12-h light/dark cycle (light on at 06:00). Food and water were available ad libitum. All animals were naïve to the current test and had no prior experience in any behavioral test. All experimental procedures were in compliance with European (ECVD 86/609/EEC) and applicable Swiss regulations.

2.2. Apparatus

Our testing apparatus was composed of an arena with a camera mounted on the top of the arena. The arena was designed to resemble animals' cage thereby minimizing animals' stress and maximizing validity of the experiment. The arena was an open-field box (length and width; 60 cm × 60 cm) covered by a pyramidal ceiling at the top. The camera was mounted at a height of about 70 cm. Arena walls were made of dark-grey-painted wood and the arena was illuminated with LED lights fixed on the ceiling. The arena was placed in a quiet testing room close to the animal room. The objects to be remembered were objects made of Lego (approx. 5 cm × 10 cm × 10 cm) in experiments 1 and 2 and metal soft drink cans (red with white writing and logo on it; approx. 6 cm diameter × 12 cm height) and a white plastic container with a red lid (approx. 7 cm × 7 cm × 10 cm) in experiment 2.

2.3. Procedure

Experiment 1. The experiment consisted of three phases: Arena familiarization; Object familiarization; and Test phase. In all phases, every animal was carried inside the nesting box to the test room which was connected to the arena using a flexible tube. The gate was opened and the animals were given the opportunity to enter and explore the arena. In all phases and in all experiments, the animals were able to freely commute between the arena and the nesting box through the connecting tube. This allowed them to return to a familiar and safe place after exploration. At the end of the experiment, the gate was closed while the animals were in the nesting box, and then animals were carried back to their cage. Pilot experiments showed that tree shrews require a lot of time to get familiar to the arena. The arena familiarization trials were considered complete when 10 min elapsed since the first time the animal had entered the arena.

After three days of familiarization to the empty arena, two identical objects were included in the arena in the object familiarization phase. Again animals could commute freely between the arena and the nesting box and now explore objects inside the arena. Every animal was allowed five minutes (starting when the animal first entered the arena) to explore objects in a single object familiarization session and video was recorded for every animal. After all trials, the arena and objects were cleaned by 70% ethanol to avoid any olfactory trails. The animals were subsequently returned to their home cage and after a 24-h retention period, one of the objects was exchanged with a novel object, and tree shrews were again brought to the arena and were allowed to explore the familiar and novel objects during the test phase. Familiar and novel objects used in this experiment were made of Lego and were counterbalanced for different animals. Every animal was allowed five minutes to navigate in the arena and freely explore objects while their behavior was recorded by the camera. Fig. 1 shows the navigation map of a tree shrew superimposed on the arena with respective objects during object familiarization and test phases. It should be noted that all phases of all experiments were conducted in a closed system and the experimenter did not handle animals or otherwise intervene during the experiment. The experimenter was monitoring animal behavior online on a computer screen through the connected camera.

Experiment 2. This experiment examined how retention is boosted by repeated exposure to familiar objects. It was conducted one month after the end of experiment 1, and was similar to the first experiment except that every animal had just one day of