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Research report

Colony formation of C57BL/6J mice in visible burrow system: Identification of eusocial behaviors in a background strain for genetic animal models of autism

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Abstract

Deficits in social interaction are primary characteristics of autism, which has strong genetic components. Genetically manipulated mouse models may provide a useful research tool to advance the investigation of genes associated with autism. To identify these genes using mouse models, behavioral assays for social relationships in the background strains must be developed. The present study examined colony formation in groups of one male and three female mice (Experiment 1) and, groups of three male mice (Experiment 2) of the C57BL/6J strain in a semi-natural visible burrow system. For adult mixed-sex colonies, 4-h observations during both the dark and light cycles for 15 days demonstrated day-dependent increases in huddling together in the chamber accompanied by decreased frequencies of active social behaviors. Sequential analyses of social interactions indicated that approaches to the back of the approached animal typically elicited flight, while approaches to the front of the approached animal failed to do so. This was seen for female to female, and for female to male approaches, as well as male to female approaches, strongly counterindicating a view that rear approach/flight specifically reflects female responsivirity to unwanted male sexual approach. For adult male colonies, similar protocols found that these social behaviors were similar to those of adult mixed-sex colonies. These findings suggest two potentially useful measures of eusocial behavior in mice, of possible value for genetic mouse models of autism; that is, huddling together and approaches to the front but not the back, of conspecifics.

Keywords: Autism model; Social behavior; Huddling; Colony formation; VBS; C57BL/6J mice

1. Introduction

Autism is a severe neurodevelopmental disorder defined by social and communication deficits and ritualistic-repetitive behaviors that are typically detectable in early childhood and continue throughout life [1,2,23]. In the original description of autism by Kanner [31], autistic children were characterized by the lack of interest in, and interactions with, others; whereas current DSM-IV criteria identify substantially more subtle deficits in reciprocal social interaction as sufficient for meeting criteria for this disorder [1]. Behavioral abnormalities in this domain in autism are varied and include such characteristics as deficits in non-verbal expression, abnormalities in the social use or understanding of language, and the presence of repetitive and stereotyped behaviors [2,41], as well as developmental deficits in social perception [56]. Although there is considerable evidence that autism has genetic components, possible genetic mechanisms of autism have not yet been identified [22,23,39,64,65].

Mouse models may provide a useful research tool to advance the investigation of genes associated with autism [16,27]. Recent advances in genetic technology have made it possible to create genetically manipulated mice that harbor very specific alterations in single genes of interest [14,19,24,36]. When the targeted gene is expressed in the brain, the behavioral phenotype of the genetically manipulated mice may reveal genetic mechanisms underlying behaviors. Mice are a relatively social species [25,37,63]. Suitable mouse models for autism would show some

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relationship to the types of social deficits that are considered core symptoms of autism, including very low levels of eusocial behaviors and deficits in maintaining peer relationships [23,41].

Genetic manipulation of mice often involves a congenic breeding strategy utilizing C57BL/6J and 129 substrains as backgrounds [16,57]. The characteristics of the background strain may have considerable relevance for interpreting results in genetically altered animals [19,24], with the possibility that specific qualitative or quantitative aspects of behavior in such mice might be determined by their genetic background rather than by the loss or overexpression of gene function [16,54].

C57BL/6J mice are a standard, commercially available inbred strain, which have used in a number of studies with a focus on social components of behavior [15,16]. However, conspecific social behaviors include approach and interactive components based on sexual and aggressive, in addition to eusocial, motivations. As sexual and aggressive motivations are not notably deficient in autistic individuals [2,41], it is important to identify behaviors that are specifically eusocial, involving purely amicable motivations.

Mouse are social animals that typically establish group or overlapping territories involving one male and several females [26,42,62]; often with shared nests that provide extensive underground burrow and tunnel complexes [11,40]. However, groups of adult mice that include multiple males plus females tend to produce high levels of male aggression with serious wounds, weight loss, and death [4,8,35,58]. In order to describe all gender combinations of social interactions in a semi-natural habitat, the present study investigated such behaviors during colony formation and maintenance in groups of one male and three female mice (Experiment 1) and in groups of three adult male mice of the C57BL/6J strain (Experiment 2). These groups were housed in large visible burrow systems (VBS) enclosures providing multiple tunnels and burrows in addition to an open "surface" area [7,9].

2. Materials and methods

2.1. Animals

Animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals [NIH, 1996]. All protocols and animal handling and treatment were approved by the Institutional Animal Care and Use Committee at the University of Hawaii.

2.1.1. Experiment 1

Four male and 12 female C57BL/6J mice, aged 8 weeks at the day of colony formation, were used as the subjects. Upon arrival from Jackson Laboratories (San Diego, CA), they were housed in polypropylene cages, 26.5 × 17 × 11.5 (H) cm, in a temperature-controlled room (22 ± 1 °C) for 1 week before the experiment. Female mice were group housed (each $N = 3$), while male mice were singly housed, with free access to food and water.

2.1.2. Experiment 2

Twenty-one male mice of the C57BL/6J strain, aged 9–12 weeks at the day of colony formation, were used as the subjects. There were offspring of dams from Jackson Laboratories (San Diego, CA). The subjects had been born and reared in polypropylene cages, 26.5 × 17 × 11.5 (H) cm, in a group of two to three male littermates after weaning at the 26 days of age, in a temperature-controlled room (22 ± 1 °C).

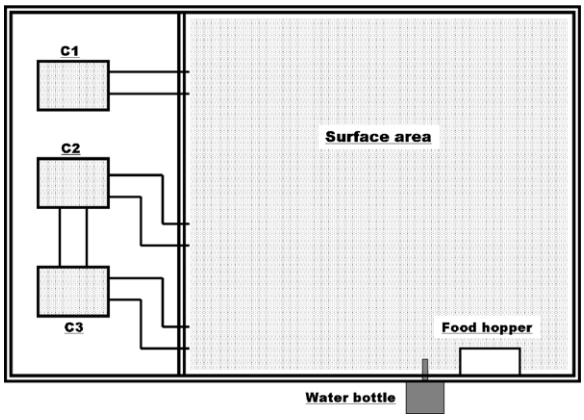

Fig. 1. A schematic diagram of the visible burrow system using in the present experiments. C: chamber, mesh pattern: a layer of sawdust bedding.

2.2. Apparatus

Each colony (VBS) was housed in a rectangular, galvanized metal bin, 86 × 61 × 26 (H) cm (Fig. 1). Three chambers, each 12 × 7 × 6 (H) cm, were positioned behind a barrier wall extending across a short width (61 cm) of the bin, 25 cm from the end wall. This wall separated an open "surface" area (the larger area of this apparatus) from the chambers in the smaller area (see Fig. 1). These chambers were connected to and opened though the wall via clear Plaxiglas tubes 5 cm in diameter. Two of the three chambers, each connected to the "surface" area via a "Z" shaped tube, were connected to each other via a straight clear Plaxiglas tube. The third chamber was connected only to the surface, via a straight tube. The animals could pass freely between each chamber and the "surface" area, or between the two connected chambers, by these tubes. Food hoppers and water tubes were located in a far corner of the "surface". All dividing walls and chambers were constructed of black Plexiglas, except the chamber tops which, along with the surface area top, were clear Plexiglas to permit videotaping. The floor was covered by a layer of sawdust bedding (1 cm) in all chambers as well as the surface. A video camera connected to a DVD recorder was mounted on the ceiling over the VBS.

The experiment room was illuminated on a 12-h light:12-h dark cycle with lights-on 07:00–19:00 h. The colony was illuminated by fluorescent lamps during the light period of the daily light-dark cycle and by infrared light in the dark period. The temperature and humidity were maintained at 22 ± 1 °C and 70% humidity.

2.3. Procedure

2.3.1. Observation of VBS colonies

Twenty-four hours prior to colony formation, subjects were marked for identification with a commercial crème-based hair dye (SALLY HANSEN DIV., DIST, extra strength crème hair bleach). On day 1, each group of four mice (one male and three females) in Experiment 1 and three male mice in Experiment 2 were moved from the rearing room to the testing room and placed in a VBS at the beginning of the dark period of the daily light-dark cycle. Four VBS colonies were scored simultaneously in the testing room. VBS colony grouping was maintained for 15 days. There were four colonies in Experiment 1 and seven in Experiment 2. All animals of a particular colony were previously unfamiliar each other. Each colony was cleaned after recording on days 3, 7, and 11. After cleaning of VBS on days 3, 7, 11, and 15 in Experiment 2, male mice were weighed in order to check their health and social dominance–subordinate relationships [9].

Video recordings were made of each colony for 4 h daily, on days 1, 2, 6, 10, and 14 in the dark cycle and days 2, 3, 7, 11, and 15 in the light cycle. Behaviors were analyzed by time sampling with an initial 30-s sample being taken every 10 min for each mouse.