NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

H. Arakawa / Neuroscience xxx (2017) xxx–xxx

RESEARCH PAPER

Analysis of Social Process in Two Inbred Strains of Male Mice: A Predominance of Contact-Based Investigation in BALB/c Mice

Hiroyuki Arakawa *

Rodent Behavioral Core, Department of Research Administration, Case Western Reserve University School of Medicine, United States

Abstract—Developing mouse models for social communication deficits requires a better understanding of the nature of social investigatory processes between mice. Mice use different investigatory strategies based on a possibility of contacts with social sources. A detailed investigation of contact distance revealed strain differences in behavioral strategy between two male inbred C57BL/6 (B6) and BALB/c (BALB) mouse strains. When direct physical contact with stimulus mice was restricted, BALB mice displayed lower social approaches than B6 mice, accompanied by heightened innate anxiety in an unfamiliar environment. However, both BALB and B6 mice expressed distinct object and social recognition in the habituation/dishabituation paradigm. When allowed direct contact with stimulus mice, both B6 and BALB mice showed approach and discrimination of strain differences in the stimulus mice. Furthermore, BALB mice discriminated individuals of the same strain among cagemates and showed a discrete aversion to the anogenital but not facial region of the stranger mice. This anogenital aversion disappeared when the stranger mice received a buspirone injection that reduced anxiety or when familiar cage mates were exposed. These strain differences in investigatory strategies illustrate that B6 mice are able to respond to and process social cues in a vicinity, which does not require physical contact with the source, while BALB mice predominantly process social cues by direct contact with the source. Although BALB mice exhibit marked anxiety and defensive responses to unfamiliarity, there is no evidence of any defect in sociability in BALB mice as a possible autism model. © 2017 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: sociability, social recognition, social contact, behavioral strategy, autism model, BALB/c.

INTRODUCTION

The survival and reproductive success of most animals relies on successful social interactions and relationships with conspecifics. Mice are a highly social species that possess a variety of social behavior and communication behaviors, providing a powerful model to study the molecular genetic basis of these behaviors and to evaluate neural mechanisms underlying a deficit in social behavior and communication as valuable sources for translational research.

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder, defined as persistent deficits in social and communicative interactions and maintenance of social relationships (Kogan et al., 2009). Various assays and measurements have been proposed and utilized for mouse models to elucidate a primary behavioral phenotype of ASD, including social signaling processes such as scent marking (Arakawa et al., 2008), ultrasonic vocalizations (Wöhr, 2014), reciprocal social interaction among juvenile mice (Ricceri et al., 2007), and sociability tests such as the three chamber social choice model (Nadler et al., 2004; Moy et al., 2004). In particular, the sociability test, also called the three-chamber test, has been widely used as a standard test for social behavior in mouse models. In the social choice paradigms, a test mouse is placed in a test chamber and can choose to approach or not approach a stimulus mouse that is confined to a restricted area of the chamber such as a wire-mesh or grid cylinder (Insel and Young, 2001; Brodkin et al., 2004). For instance, the BALB/c (BALB) and C57BL/6 (B6) inbred mouse strains are characterized by low and high sociability, respectively, as assessed by a greater amount of social approach in B6 mice, compared with more social avoidance in BALB mice among several inbred strains in this preference chamber (Brodkin et al., 2004; Sankoorikal et al., 2006). In this test,

*Address: Department of Research Administration, Case Western Reserve University School of Medicine, 2109 Adelbert Road, ARC-RB05P, Cleveland, OH 44106, United States.
E-mail address: hxa171@case.edu

Abbreviations: ANOVA, analysis of variance; ASD, autism spectrum disorder; SEM, standard error of the mean.

https://doi.org/10.1016/j.neuroscience.2017.10.045

however, the stimulus mice cannot express a behavioral reaction and thus directly contact the test mouse.

Sequential analyses of communicative behaviors in mice have illustrated a more profound, complicated process between the test and stimulus mice (Doty, 1986; Hurst and Beynon, 2004). The sensory communication of nocturnal mice heavily relies on the olfactory sense (Welker, 1964; Brown and Macdonald, 1985). Mice initially exhibit an approach behavior when they have detected some unfamiliar social cue such as airborne volatile odorants or vocalization from a distance (Hurst and Beynon, 2004). Detection of the first social cue does not require physical contact with the social source. The social approach, or sociability assessed by the social choice paradigm is measured at this time point and thus depends on auditory and volatile-based olfactory cues emitted by the stimulus mice. When the test mouse decides to approach the social source, *viz.* a stimulus mouse, they closely investigate it to obtain more information via physical contacts such as nonvolatile odorant cues involving individual genetic identification (Halpin, 1986; Nevison et al., 2000). Mice typically investigate the facial area of the stimulus mouse, where they sniff and taste facial excrements released from the nose, palatum, and exocrine glands such as the lacrimal gland and the salivary gland (Haga et al., 2010; Arakawa et al., 2011), and touch whiskers with whiskers (Welker, 1964; Hartmann, 2011). Anogenital sniffing is frequently observed when mice contact with the stimulus mouse, in which mice thoughtfully sniff the anogenital region of the stimulus mice to gather further information from the exocrine and secretory fluid (Blanchard and Blanchard, 1977; Wesson, 2013). As a result, the stimulus mice respond to these approaches and display a variety of social behaviors including counter-sniffing, flight response, and aggressive bouts (Blanchard and Blanchard, 1977; Doty, 1986).

BALB mice have been proposed as an animal model of ASD since they typically show a heightened social avoidance in the social choice paradigm (Brodkin et al., 2004; Sankoorikal et al., 2006). BALB mice are also known to be highly anxious in a variety of anxiety test paradigms such as the open-field or elevated plus maze (Belzung and Griebel, 2001; Bouwknecht and Paylor, 2002), and highly aggressive compared with other inbred strains (Southwick and Clark, 1968; Mondragón et al., 1987). A potential concern is that the low social approach observed in BALB mice may be partially due to their high innate anxiety level in a novel environment or increased aggressiveness to conspecifics. Furthermore, a recent study demonstrated that BALB mice are incapable of recognizing a social cue that is associated with familiarity emitted by conspecifics from a distance (Arakawa, 2017). Familiarity-related cues induce a social approach in B6 mice (Arakawa et al., 2015), suggesting that a lack of recognition of these familiarity cues in a vicinity may be partly responsible for the reduced social approach of BALB mice in the social choice paradigm.

To assess the details of the social process including the distance approach and subsequent physical contacts, we observed the behavior of BALB mice relevant to the standard social strain, B6, in a social preference setting (Exp1), social recognition setting (Exp2), and physical interaction setting (Exp3). To elucidate whether the mice show particular investigatory patterns to different body parts of the opponents (facial vs. anogenital area), we tested investigatory patterns of the mice to restraint stimulus mice (Exp4). Moreover, we assessed whether the mice showed differential preferences to bodily parts (facial vs. anogenital area) of the opponents with different familiarity (stranger, stranger with buspirone injection, vs. cagemate) (Exp5). This final experiment was crucial because social olfactory cues released from different body parts depend on familiarity and are key determinants of social investigatory behavior.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6J mice and male BALB/cJ mice as the subjects and male 129/SvJ and DBA/2J mice as the stimulus animals were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and maintained in the colony room of the facility in the Case Western Reserve University School of Medicine. The colony room was temperature-controlled at 23 °C, with a humidity of approximately 55% under a 12-h light–dark cycle (lights on at 6:00 a.m.). Mice were maintained in standard shoe-box cages (26.5 × 20 × 16.5 height cm) with water and food provided ad libitum. All mice were housed in groups of three to four of the same sex and strain. Behavioral tests were performed when subjects and stimulus mice reached an age of at least 12 weeks. Juvenile stimulus mice were used at 3–4 weeks of age. All test trials were conducted during the light phase of the light/dark cycle under dimly lit conditions. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and use of Laboratory Animals (2011) and approved by the Case Western Reserve University School of Medicine Institutional Animal Care and Use Committee.

Experimental design

Experiment 1: (Social preference test): Male subject mice (B6, $n = 24$; BALB, $n = 24$) and male stimulus mice (129 or DBA each $n = 6$) were used for the social preference test. They were randomly assigned to two experimental groups: habituated vs. non-habituated (each $n = 12$). Then the subject mice were confronted with a stimulus mouse of either 129 or DBA strain. The sample size of 12 has a 90% of detecting strain differences at a 5% two-tailed significance level. We decided to use the sample size of 12 for all the tests listed herein.

Experiment 2: (Social recognition test): Male B6 and BALB mice ($n = 12$ each) were used as the subjects and also as the stimulus animals of cagemate conditions ($n = 12$ each). Male 129 and DBA mice, juvenile ($n = 12$ each) or adult ($n = 12$ each) were used as the stimulus animals (stranger).

Please cite this article in press as: Arakawa H. Analysis of Social Process in Two Inbred Strains of Male Mice: A Predominance of Contact-Based Investigation in BALB/c Mice. Neuroscience (2017), https://doi.org/10.1016/j.neuroscience.2017.10.045