Social Behavior Tests for Mice

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Mice Are a Social Species

If you are interested in the biology of social behaviors, are generating mouse models of psychiatric disorders, or simply enjoy watching and understanding animal behavior, you are in luck. Many excellent assays of mouse social behaviors are well-established in the behavioral neuroscience and behavioral neuroendocrinology literature. The references listed below include enlightening reviews and step-by-step protocols. This overview will briefly describe some of the most commonly used methods for quantitating social interaction, affiliation, sexual behaviors, parental behaviors, juvenile play, social dominance, aggression, social recognition, social memory, and social communication in mice. These tasks apply to phenotyping mouse models of human social dysfunctions, such as those found in autism, schizophrenia, aggression, and social phobias.

Social Interactions in Mice

Two unfamiliar mice placed in a neutral arena will usually display high levels of sniffing, following, crawling over and under each other, nose-tonose sniffing, and anogenital sniffing (Fig. 1A). Allogrooming, in which one mouse grooms the other, is frequently observed in a neutral arena and when two or more mice reside in the same home cage. Scoring of social interactions is commonly performed using videotapes of social interaction sessions in the home cage, empty novel cage, structured novel environment, or in an open-field chamber. A freestanding digital video camera or a computer-assisted video tracking system is used to record and store an electronic version of the session for subsequent analysis. The researcher scores the video sessions for frequency and duration of carefully defined behavioral events. Scoring can be conducted using either pencil and paper, a keyboard event recorder, or an automated video tracking software system. Automated and semiautomated systems that measure social approach and social recognition usually calculate the number of approaches, total time spent together, and specific components of interaction by each individual (Kwon et al., 2006; McFarlane et al., 2007) (Fig. 1B). Each behavioral parameter is analyzed independently using the appropriate statistical tests. For some purposes, a composite score of total social interactions is employed (Bolivar et al., 2007). On most parameters of social interaction, juvenile mice tested at 20–25 days of age display play behaviors that resemble adult social interactions (Terranova and Laviola, 2005; Panksepp and Lahvis, 2006; McFarlane et al., 2007).

Aggressive Behaviors

Dominance hierarchies are common among groups of male mice. For example, introducing a new male mouse into the home cage of an unfamiliar adult male mouse is likely to lead to aggressive behaviors. The standardized resident-intruder test is used to score tail rattling, following behaviors, latency to first attack, number of attacks, duration of fighting, and body scars (Miczek et al., 2001). A second approach used to quantitate aggressive tendencies is isolation-induced fighting. Isolating male mice in individual housing cages for several weeks will result in high levels of attack and fighting when the isolated males are subsequently placed together in a test arena (Valzelli et al., 1974). Fighting is more common among male mice than female mice (Compaan et al., 1993; Miczek et al., 2001). If it is important to avoid actual fighting and scarring, the tube test is a good choice for measuring dominant-subordinate status. Two male mice are placed in a cylindrical plastic tube, and the individual that retreats to one end is scored as the subordinate (Hahn and Schanz, 1996; Spencer et al., 2005).



Figure 1. *A*, Nose-to-anogenital sniffing is commonly seen when unfamiliar mice are placed together. Olfactory pheromones in the anogenital area are detected by the vomeronasal organ as cues used for social recognition in rodents (Keverne, 2002). *B*, Automated 3-chambered social approach task, with photocells embedded in the openings between chambers, tallies the amount of time the subject mouse spends in the middle start chamber, the side chamber containing a wire cup novel object, and the side chamber containing a new mouse (Moy et al., 2007; McFarlane et al., 2007).

Photographs by Janet Stephens, NIH Photography, and the author.

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Sexual Behaviors

Introducing two normal adult mice of the opposite sex into the same cage is likely to lead to sexual behaviors. Males are scored for following, sniffing, mounting, thrusts, and intromissions (Scordalakes et al., 2002). Females are scored for lordosis and subsequent presence of a vaginal plug indicating insemination (Keller et al., 2006). Well-validated and standardized methods are available for conducting surgical ovariectomy, followed by hormone implants and injections to regulate receptivity and estrus in the females (Sisk and Meek, 1997), and for scoring sexual behaviors (Sisk and Meek, 1997; Scordalakes et al., 2002; Keller et al., 2006).

Parental Behaviors

Both male and female mice contribute to parenting. Both parents build nests and huddle with their pups (Bult and Lynch, 1996; Lijam et al., 1997; Moretti et al., 2005). Nests are scored for height, shape, quality, and utilization. Maternal care is scored for licking, sitting with, crouching, nursing, and retrieving the pups. When a pup strays from the nest or is removed by the experimenter, the pup emits ultrasonic vocalizations (Branchi et al., 2001; Hofer et al., 2001). Both parents locate the calling pup and retrieve it, returning it to the nest.

Social Recognition, Preference, and Memory

Individual recognition is interpreted to have occurred when the subject mouse displays more investigation of an unfamiliar mouse and less investigation of a familiar mouse upon repeated exposures to these conspecifics. The observer scores time spent in social interactions during brief exposures sessions, e.g., for 5 minutes. Preference for a specific individual, gender, strain, or genotype is demonstrated when the subject mouse spends more time interacting with one individual than with another in a choice test (Winslow, 2003) (Fig. 2A). Preference for social novelty is demonstrated when the subject mouse spends more time with a new mouse than with a familiar mouse (Crawley et al., 2007; Moy et al., 2007) (Fig. 2B). Social memory is evaluated by inserting a time delay, e.g., 30 minutes, between repeated exposures to the same and different mice (Ferguson et al., 2000).

Social Communication

Olfactory

Most communication between mice appears to employ olfaction (Keverne, 2002). Urine deposits elicit high levels of investigative sniffing. Interest in urine scents is measured in terms of the frequency and duration of sniffs directed at urine, which is delivered through various means, including an olfactometer delivering a stream of volatile odors into a port in an operant chamber, or cotton swabs soaked in urine (Wersinger et al., 2006). The olfactory habituation/ dishabituation test employs cotton-tipped applicator swabs soaked in nonsocial and social odors, such as water, almond, banana, lemon, mouse urine, and floor wipes from soiled mouse cages (Luo et al., 2002; Wrenn et al., 2003; Wersinger et al., 2006; Crawley et al., 2007) (Fig. 3A). Olfactory communication of new food flavors on the breath of a cage mate is measured using the social transmission of food preference test (Wrenn et al., 2003; Wrenn 2004; McFarlane et al., 2007) (Fig. 3B)



Figure 2. *A*, Social choice test (from Winslow, 2003). The subject mouse in the center chamber walks through the cylindrical tunnel to interact with one of the two tethered target mice. *B*, Preference for social novelty test. The subject mouse in the center chamber walks through the doorways to spend time interacting with either a familiar mouse in one side chamber or an unfamiliar mouse in the other side chamber. Photocells embedded in the panels around the doorways count time spent in each chamber and number of entries.

Photographs by Ms. Selen Tolu, NIMH, and the author.

Auditory

Auditory communication among mice is an emerging research field. Complex vocalizations are emitted by juveniles engaged in social interactions and by adult males in response to female pheromones (Panksepp et al., 2007; Guo and Holy, 2007). As described above, ultrasonic vocalizations emitted by separated pups serve as distress calls the parents use to detect and locate the pup and retrieve it, bringing it back to the nest (Winslow et al., 2000; Hofer et al., 2001; Branchi et al., 2001) (Fig. 3C). Maternal potentiation of pup ultrasonic vocalizations is a modification that may incorporate more cognitive components. Rat pups twice separated from the dam display more ultrasonic vocalizations during the five minutes after the second separation than during the five minutes after the first separation (Hofer et al., 2001). There is some evidence for maternal potentiation of ultrasonic vocalizations in mice (Moles et al., 2004; M.L. Scattoni and J.N. Crawley, NIMH, unpublished

observations). Since the separations are identical in every other way, and occur five minutes apart, it seems likely that the pup is regulating its response based on a cognitive interpretation of its previous separation experience. Intentionality and functional significance of mouse ultrasonic vocalizations remain to be determined. Studies are needed that feature playback tape recordings of salient vocalizations in mice, similar to those studies used to investigate vocal communication among birds (Konishi, 2004).

Motivation for Social Interactions

Another area of mouse social behaviors that requires new ideas is the measurement of motivational level for engaging in social interactions. Conditioned place preference for the chamber in which a social partner was previously located has been validated as a test for social reinforcement among rats as well as among juvenile C57BL/6J, A/J, and DBA/2J inbred strains of mice (Everitt, 1990; Panksepp and Lahvis,



Figure 3. Social olfactory tests. *A*, Olfactory habituation/dishabituation. An observer measures the time spent by the subject mouse in sniffing new and familiar social smells, such as mouse urine or cage swipes, presently sequentially. Photograph by Janet Stephens, NIH Photography, and the author. *B*, Social transmission of food preference. (1) One cage mate (demonstrator) consumes a novel flavored food and (2) communicates the odor on its breath to its cage mates (observers). The observer eats more of the flavored food detected on the mouth and whiskers of the demonstrator than it does a completely new flavored food. Photographs by Valerie Bolivar, Wadsworth Center, Troy, NY; diagram by Valerie Bolivar, Wadsworth Center, Troy, NY, modified by Hewlet McFarlane, Kenyon College, Gambier, OH. Adapted from McFarlane et al., 2007. *C*, Ultrasonic microphone in the lid of a Styrofoam box records ultrasonic vocalizations emitted by a mouse pup separated from its nest. Photograph by Janet Stephens, NIH Photography, Dr. Maria Luisa Scattoni, NIMH, and the author.

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2006). Maternal motivation for retrieving rat pups is measured in terms of responses of the mother using an operant lever to deliver pups from a carousel (Lonstein and Fleming 2001) (Fig. 4A). A rat operant chamber has been modified to allow the investigator to open a trapdoor and deliver a social partner when the subject rat presses a lever on a fixed ratio schedule (Everitt, 1990) (Fig. 4B). Automated twochamber systems with electronic access doors that open and close according to a reinforcement schedule are needed in order to allow researchers to quantitate the number of nose-pokes a mouse is willing to make to gain access to a social partner.

Mouse Models of Aberrant Social Behaviors

Aberrant social behaviors or low levels of social interaction are symptoms of several psychiatric disorders, including autism, anxiety, depression, schizophrenia, and social phobias. Genetic, pharmacological, and lesion models of neuropsychiatric disorders are increasingly available. In these models, transgenic and knockout mice with mutations in candidate genes for a disease are phenotyped for behavioral traits with face validity, i.e., conceptual analogy to the human symptoms (Crawley, 2007). Some of the first laboratory protocols for measuring social interactions in rodents came from studying models of anxiety. Two rats placed in an unfamiliar environment will display less sniffing and following behavior under high levels of illumination than when the ambient light is dim (File and Hyde, 1978). Anxiolytic drugs increase social interaction in the brightly lit arena (File, 1997). Social cognition deficits in schizophrenia could be modeled using some of the tests described above. It is interesting to speculate that the social anhedonia seen in some forms of depression, in which the patient gains no pleasure from engaging in social interactions and avoids social environments, could be modeled using some of the social motivation tasks described above.

Autism is diagnosed on the basis of aberrant social interactions as well as impaired communication and repetitive behaviors. Given the strong genetic component to autism spectrum disorders, mouse models of autism are increasingly focusing on knockouts of candidate genes that have been identified in human association studies, as well as inbred strains with unusual background genes that may be relevant to social behaviors (Crawley, 2004). Measures of social interactions between mice tested in a variety of ways offer complementary approaches for quantifying abnormal levels of sociability. Examples include mice tested in an empty arena (Spencer et al., 2005; Bolivar et al., 2007), in a 3-chambered apparatus (Winslow, 2003;



The Pupomat: An operant task for pup reinforcement



Figure 4. Social motivation equipment. *A*, Pupomat carousel delivers a pup when the mother presses a lever (from Lonstein and Fleming, 2001). *B*, Male rat presses a lever on a fixed ratio schedule to gain access to a familiar female rat located above the operant chamber.

Photograph kindly contributed by Professor Barry Everitt, University of Cambridge, UK; adapted from Everitt, 1990.

Brodkin, 2007; Moy et al., 2007; McFarlane et al., 2007) (Figs. 1B, 2A, 2B), in a visible burrow (Arakawa et al., 2007), in a socially conditioned placepreference chamber (Everitt, 1990; Panksepp and Lahvis, 2006), and using video tracking systems (Kwon et al., 2006).

Aberrant forms of reciprocal social interactions detected in mouse models of autism, Rett syndrome, © 2007 Crawley fragile X syndrome, schizophrenia, and other disorders characterized by social deficits provide translational phenotypes for testing hypotheses about biological mechanisms and for evaluating the therapeutic efficacy of proposed treatments.

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