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Alterations in grooming activity and syntax in heterozygous SERT and BDNF knockout mice: The utility of behavior-recognition tools to characterize mutant mouse phenotypes

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ABSTRACT

Serotonin transporter (SERT) and brain-derived neurotrophic factor (BDNF) are key modulators of molecular signaling, cognition and behavior. Although SERT and BDNF mutant mouse phenotypes have been extensively characterized, little is known about their self-grooming behavior. Grooming represents an important behavioral domain sensitive to environmental stimuli and is increasingly used as a model for repetitive behavioral syndromes, such as autism and attention deficit/hyperactivity disorder. The present study used heterozygous ($^{+/-}$) SERT and BDNF male mutant mice on a C57BL/6J background and assessed their spontaneous self-grooming behavior applying both manual and automated techniques. Overall, SERT$^{+/-}$ mice displayed a general increase in grooming behavior, as indicated by more grooming bouts and more transitions between specific grooming stages. SERT$^{+/-}$ mice also aborted more grooming bouts, but showed generally unaltered activity levels in the observation chamber. In contrast, BDNF$^{+/-}$ mice displayed a global reduction in grooming activity, with fewer bouts and transitions between specific grooming stages, altered grooming syntax, as well as hypolocomotion and increased turning behavior. Finally, grooming data collected by manual and automated methods (HomeCageScan) significantly correlated in our experiments, confirming the utility of automated high-throughput quantification of grooming behaviors in various genetic mouse models with increased or decreased grooming phenotypes. Taken together, these findings indicate that mouse self-grooming behavior is a reliable behavioral biomarker of genetic deficits in SERT and BDNF pathways, and can be reliably measured using automated behavior-recognition technology.

1. Introduction

Serotonin transporter (SERT; 5-HTT) and brain-derived neurotrophic factor (BDNF) are key modulators of brain development and function (Huang and Reichardt, 2001; Murphy and Lesch, 2008). SERT is responsible for the re-uptake of serotonin from the synaptic cleft to presynaptic neurons. In humans, alterations in the SERT gene (*SLC6A4*) are implicated in multiple neuropsychiatric disorders, including anxiety, depression, obsessive–compulsive disorder (OCD), autism and attention deficit/hyperactivity disorder (ADHD) (Hu et al., 2006; Karg et al., 2011; Lesch et al., 1996; Murphy et al., 2004; Murphy and Lesch, 2008; Sen et al., 2004). Although SERT represents one of the most widely studied genes, the exact biological mechanisms underlying these associations remain unclear (Murphy et al., 2004; Murphy and Lesch, 2008). In addition to homozygous SERT$^{-/-}$ rats and mice (which display overt developmental and behavioral deficits; Holmes et al., 2003b; Homberg et al., 2007; Kalueff et al., 2010; Murphy and Lesch, 2008), SERT$^{+/-}$ rodents also show altered emotional and motor behaviors, as well as increased sensitivity to various experimental manipulations (Ansorge et al., 2004; Fox et al., 2007; Moya et al., 2011; Murphy and Lesch, 2008). Their 50% decrease in transporter activity (Fox et al., 2009; Snoeren et al., 2010) resembles polymorphisms in the human SERT gene (Hu et al., 2006; Lesch et al., 1996; Maurex et al., 2010; Praschak-Rieder et al., 2007), especially the well-studied human SERT-linked promoter region (5HTT-LPR, consisting of the 'active' L allele and the 'less active' S allele), which is strongly implicated in multiple behavioral syndromes (Blom et al., 2011; Kuzelova et al., 2010; Nikolas et al., 2010).

BDNF is crucial for various brain processes, including cell differentiation and survival, axonal growth, neurogenesis and memory formation (Acheson et al., 1995; Bekinschtein et al., 2008; Cheng

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et al., 2011; Pencea et al., 2001), acting via tyrosine kinase B (TrkB) and p75 receptors. The most common variant in the human BDNF gene is the substitution of valine for methionine at codon 66 (Val66Met), which impairs intracellular trafficking and secretion of BDNF (Chen et al., 2004) and is implicated in schizophrenia, depression, anxiety, substance abuse, Parkinson's disease and cognitive deficits (Chao et al., 2008; Colzato et al., 2011; Kanellopoulos et al., 2011; Karakasis et al., 2011; Matsuo et al., 2009; Savitz et al., 2006). While BDNF$^{-/-}$ mice are not viable, BDNF$^{+/-}$ mice have long been used in neuroscience research, showing altered emotionality, neurophysiology and neuromorphology (Bartoletti et al., 2002; Kernie et al., 2000; Lyons et al., 1999; MacQueen et al., 2001; Zhu et al., 2009). BDNF also interacts with SERT at the molecular level, modulating its release and synthesis (Benmansour et al., 2008; Deltheil et al., 2008b; Molteni et al., 2010), whereas serotonin levels, in turn, influence BDNF secretion and mRNA expression (Allaman et al., 2011; Deltheil et al., 2008a,b).

Although SERT and BDNF mutant mice have been extensively evaluated in various experimental paradigms, there are no systematic studies of several important behavioral domains, including self-grooming. Representing an evolutionarily conserved behavior highly sensitive to various genetic, environmental and pharmacological manipulations (Colbern and Twombly, 1988; Kalueff and Tuohimaa, 2005a; Sachs, 1988), self-grooming is the most common waking rodent behavior, and its translational significance is increasingly appreciated in biological psychiatry (Fineberg et al., 2011; Mehta et al., 2011; Silverman et al., 2010).

Rodent grooming generally follows a fixed pattern, progressing in a cephalo-caudal (paws/nose to tail/genitals) direction (Berridge et al., 2005; Fentress, 1988), which itself is bidirectionally sensitive to anxiety and stress (Kalueff and Tuohimaa, 2004, 2005b). Regulated by the hypothalamus and basal ganglia, this complex and patterned behavior is an appropriate phenotype to study in various behavioral syndromes, including anxiety, OCD, autism, ADHD and substance abuse (Aldridge et al., 2004; Kruk et al., 1998; Ming et al., 2007; Mink and Thach, 1993; Rapoport and Wise, 1988). Given the importance of grooming for both rodent and human behaviors (including psychiatric disorders already linked to SERT and BDNF; Berridge et al., 2005; Graybiel and Saka, 2002; Welch et al., 2007), this phenotype merits further scrutiny using genetic mouse models with altered SERT and BDNF function. Combining sophisticated grooming analysis protocols (Kalueff et al., 2007a; Kalueff and Tuohimaa, 2004, 2005a) and recently developed tools for the automated high-throughput phenotyping of mouse grooming (Kyzar et al., 2011), this study examines the activity and patterning (behavioral organization) of grooming behaviors in SERT$^{+/-}$ and BDNF$^{+/-}$ mice.

2. Methods

2.1. Animals

The present study used 36 adult male (5–8 months old) SERT$^{+/-}$ and BDNF$^{+/-}$ mice and their wild type ($^{+/+}$) C57BL/6J counterparts ($n = 9$ per group), originally obtained from Jackson Laboratory (Bar Harbor, ME) and housed 4–5 mice per cage with free access to food pellets and water. SERT$^{+/-}$ mice were chosen for this study because their ~50% decrease in SERT activity (Kim et al., 2005) mimics the molecular phenotype of human SERT polymorphisms associated with multiple psychiatric disorders (Canli et al., 2006; Caspi et al., 2003; Murphy et al., 2004). BDNF$^{+/-}$ mice were chosen because of their viability compared to BDNF$^{-/-}$ mice, and the association of various psychiatric conditions with BDNF dysfunction (Angelucci et al., 2004; Craddock and Forty, 2006; Fontenelle et al., 2012; Martinowich and Lu, 2008; Nishimura et al., 2007; Yoshimura et al., 2010). Prior to testing, the mice were transported from their holding room to the testing room and allowed at least 1 h for acclimation. All observations were part of animal coat state and welfare inspection and were performed between 11:00 and 15:00 to ensure uniformity throughout the trials. Animals were individually placed in a clear observation cylinder (13 cm in diameter, 15 cm height) for behavioral observation as part of regular animal inspection. To assess spontaneous 'novelty evoked' grooming, the mice were video-recorded by a side-view web camera (LifeCam Cinema HD, Microsoft Corp., Redmond, WA) and manually analyzed for 5 min, similar to Kyzar et al. (2011). The observation cylinder was thoroughly cleaned using 70% ethanol (vol/vol) between subjects.

2.2. Behavioral analyses

2.2.1. Grooming analysis

During manual scoring, two highly trained observers (intra- and inter-rater reliability > 0.85, as determined by Spearman correlation) used the Grooming Analysis Algorithm (Kalueff and Tuohimaa, 2004) to record the latency, direction and duration of each grooming bout and its constitutive episodes (paw licks, head washes, body/leg washes and tail/genital washes), as described previously (Kalueff et al., 2007a; Kyzar et al., 2011). A grooming "bout" was characterized as continuous self-grooming without interruption (defined as a full stop in grooming action for more than 3 s). An "episode" was identified as a portion of a single bout in which the subject is grooming a specific body region (e.g., paw licks and body/leg washes), and a "transition" was defined as a progression from one grooming episode to another separate episode within a single grooming bout, according to Kyzar et al. (2011). Rostral grooming consisted of paw licking and head wash behavior, while caudal grooming included as body/leg wash and tail/genital wash behavior.

The videos were analyzed using the HomeCageScan software (CleverSys, Inc., Reston, VA) which recognizes and detects rodent movements and behaviors based on video-tracking of multiple individual body parts, posture and frequency of movements (Kyzar et al., 2011; Liang, 2010) (Fig. 1). While complete grooming bouts often culminate in tail and genital washes, these were not quantified in the automated portion of this study due to the difficulty with distinguishing these grooming behaviors from body/leg washes within the existing software (see Kyzar et al., 2011 for details). To optimize grooming detection, we applied customized settings to detect only grooming bouts (<3 s) lasting longer than 3 s, reducing false positives associated with the detection of relatively rare extra-short bouts (generally representing <5% of grooming activity; Kyzar et al., 2011). To ensure reliability between detection techniques, manually scored extra-short grooming bouts (< 3 s) were also not assessed here. The detection settings were specifically upgraded by the manufacturer for this study, enabling the software to distinguish between different episodes of grooming and to detect transitions between them (Kyzar et al., 2011). Additionally, recognition features which facilitated the detection of paw licking, head washing and body/leg washing behaviors were added by the developers to the existing software package specifically for this study (Kyzar et al., 2011).

The HomeCageScan software uses whole body and individual body part features, as well as grooming magnitude information, during an on-going grooming bout to perform the classification in real time (Kyzar et al., 2011). A set of rule-based tests is used by the software to determine a likelihood value for each preset category within a given segment. The category with the highest likelihood for that episode is elected as the "winner" to be recorded as the software output. The program generates an output containing all of the episode classifications for a given subject at the end of each trial (Kyzar et al., 2011). Finally, to increase detection reliability, each bout registered by HomeCageScan was independently verified by a highly trained observer. This ensured that each bout registered by the program was, in fact, a representative grooming behavior, thereby eliminating false positives and allowing for a more complete and accurate analysis of mouse grooming phenotypes.

2.2.2. Non-grooming analysis

To characterize non-grooming activity in all genotypes, manual observers recorded the number of vertical rears (both protected and unprotected) for each mouse during the 5 min observation session. A protected rear ("wall lean") was defined as any movement in which the mouse placed either of its front limbs on the side of the cylinder and simultaneously reared up on its hind legs. An unprotected rear ("vertical rear") represented any movement in which the mouse reared on its hind legs without placing a paw on the side of the cylinder. Defecations (number of fecal boli deposited during the test) were also recorded as a measure of autonomic function and anxiety. Finally, the videos shot for HomeCageScan analysis were also analyzed using the Ethovision XT7 (Noldus IT, Wageningen, Netherlands) software package, generating automated data on the distance traveled (m), average velocity (m/s), turning angle (°), turning rate, turning bias and meandering (°/m) for each mouse.

2.3. Statistical analyses

After each video was analyzed, the computer-generated data on the total number of grooming episodes and bouts, the duration of grooming, and the number of transitions between grooming episodes was compared to the manually scored data using the Spearman's rank correlation test to establish the reliability of software-detected vs. observer-detected scores. Data was also generated for the percentage of rostral vs. caudal grooming and the percentage of correct vs. incorrect transitions. A correct transition was defined as following the typical cephalo-caudal progression (i.e., paw lick > head wash > body/leg wash > tail/genital grooming). For example, a transition from paw licking to head wash would be scored as a correct transition, while a transition from body/leg wash to paw licking would be scored as incorrect (see details in Kalueff and Tuohimaa, 2004). Non-grooming endpoints (see above) were also generated for this study. For each