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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

Evan J. Kyzar[a], Mimi Pham[a], Andrew Roth[a], Jonathan Cachat[a], Jeremy Green[a], Siddharth Gaikwad[a], Allan V. Kalueff[a,b,*]

[a] Department of Pharmacology and Neuroscience Program, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA
[b] ZENEREI Institute, 309 Palmer Court, Slidell, LA 70458, USA

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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

* Corresponding author at: Department of Pharmacology, SL-83, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA.
Tel.: +1 504 988 3354; fax: +1 504 988 3354.
E-mail address: avkalueff@gmail.com (A.V. Kalueff).

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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

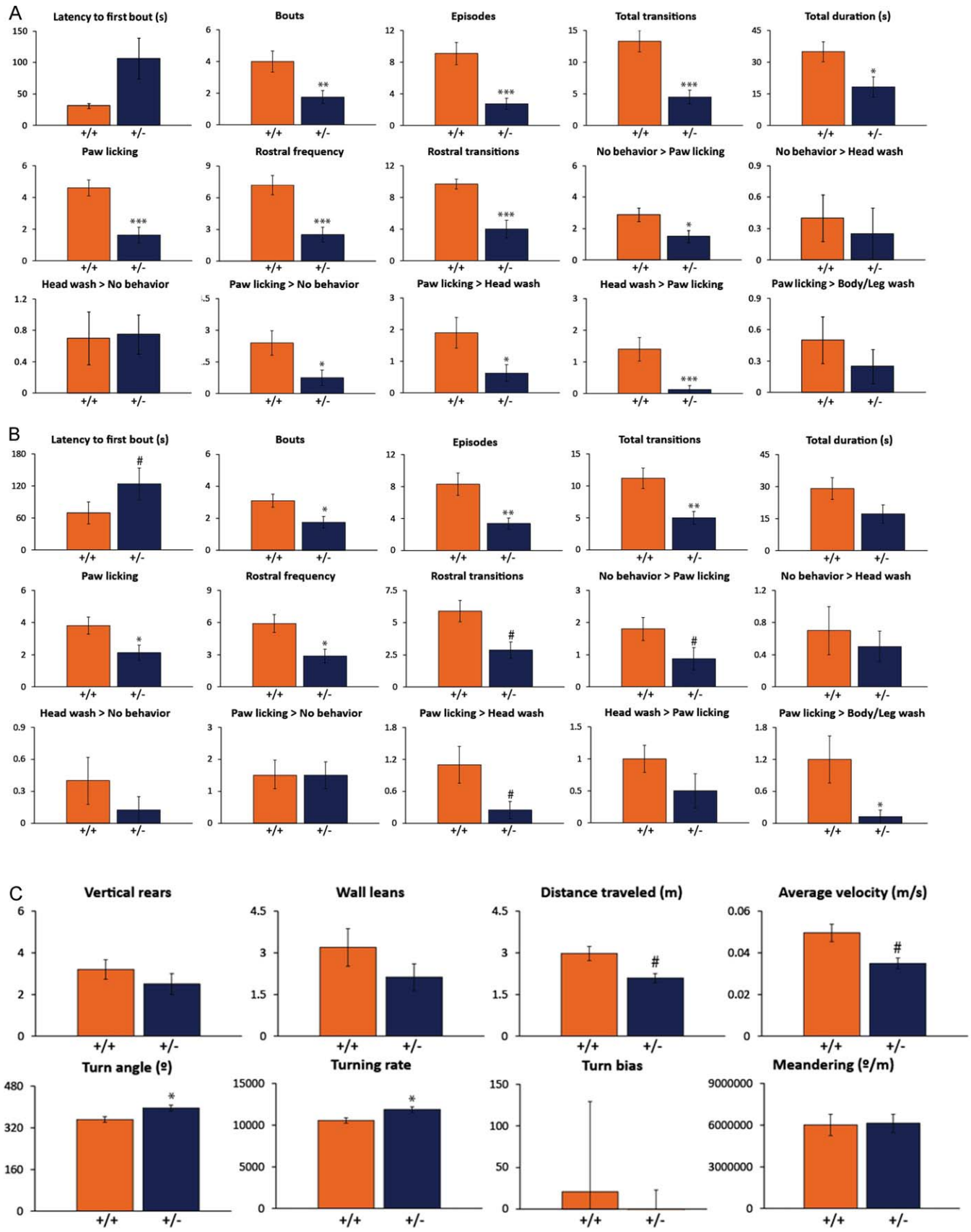

Fig. 1. Grooming and non-grooming behavior of SERT mutant mice. (A) Manual analysis of grooming behavior. (B) Automated analysis of grooming behavior as performed by HomeCage Scan software (CleverSys, Inc.). (C) Analysis of non-grooming behavior performed by manual observers (vertical rears and wall leans) and EthoVision XT7 software (Noldus IT). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, $^{\#}p = 0.05–0.01$ (trend); *U*-test vs. wild-type mice ($n = 9$ per group).

Table 1
Additional manual and automated SERT grooming and non-grooming endpoints. For manual analysis, zero values were seen in both groups for tail/genital wash frequency, "no behavior to tail/genital wash", "paw licking to body/leg wash", "paw licking to tail/genital wash", "head wash to tail/genital wash", "body/leg wash to paw licking", "body/leg wash to head wash", "body/leg wash to tail/genital wash", "tail/genital wash to no behavior", "tail/genital wash to paw licking", "tail/genital wash to head wash" and "tail/genital wash to body/leg wash" transitions. Transitions containing a "tail/genital wash" component were only characterized manually, due to the current difficulty of distinguishing this episode in the HomeCageScan software. All data is presented as mean ± SEM.

Endpoint	+/+	+/−
Manual analysis		
Head wash frequency	1.6 ± 0.4	2.4 ± 0.6
Body/leg wash frequency	0 ± 0	0.4 ± 0.4
Caudal episode frequency	0 ± 0	0.4 ± 0.4
Caudal transition frequency	0 ± 0	0.7 ± 0.7
Relative % of caudal episodes	100 ± 0	97 ± 3.0
Relative % of rostral episodes	0 ± 0	3.0 ± 3.0
No behavior > body/leg wash	0 ± 0	0.2 ± 0.2
Head wash > body/leg wash	0 ± 0	0.2 ± 0.2
% correct transitions	51 ± 5.0	48 ± 3.0
Defecations	1.8 ± 0.5	1.6 ± 0.6
Automated analysis		
Head wash frequency	0.7 ± 0.3	2.3 ± 1.0[#]
Body/leg wash frequency	0.4 ± 0.3	1.0 ± 0.6
Caudal episode frequency	0.4 ± 0.3	1.0 ± 0.6
Caudal transition frequency	0.7 ± 0.6	1.1 ± 0.6
Relative % of caudal episodes	14 ± 9.0	9.0 ± 4.0
Relative % of rostral episodes	75 ± 13	91 ± 4.0
No behavior > body/leg wash	0.2 ± 0.2	0.7 ± 0.4
Paw licking > body/leg wash	0.1 ± 0.1	0.1 ± 0.1
Head wash > body/leg wash	0.1 ± 0.1	0.2 ± 0.1
Body/leg wash > paw licking	0 ± 0	0.4 ± 0.3
Body/leg wash > head wash	0 ± 0	0.1 ± 0.1
% correct transitions	43 ± 7.0	42 ± 6.0

[#] $p = 0.05–0.01$ (trend); *U*-test vs. wild-type mice ($n = 9$ per group).

strain, heterozygous mutant mice were compared to their respective wild type controls using the unpaired Wilcoxon–Mann–Whitney *U*-test. Inter- and intra-rater reliability of observers was determined using Spearman correlation. Significance was set at $p < 0.05$ in all experiments of this study.

3. Results

3.1. Grooming and non-grooming behavior of SERT mutant mice

Using manual observation, we found significant genotype differences for multiple grooming endpoints in SERT$^{+/-}$ mice vs. their wild-type littermates, including significantly more grooming bouts, episodes and total transitions (Fig. 1A). SERT$^{+/-}$ mice also showed increased paw licking behavior, rostral grooming and rostral transitions frequency (Fig. 1A). For specific transitions, SERT mutants exhibited more "no behavior to paw licking" transitions, and a non-significant trend toward decreased latency to groom. There were no differences between the two genotypes in total grooming duration, correct vs. incorrect transitions, the percentage of caudal vs. rostral grooming episodes, or any other specific grooming transitions (Table 1).

These responses were confirmed by automated, software-generated data, as SERT$^{+/-}$ mice displayed significantly more grooming bouts, episodes, total duration, total transitions, and decreased latency to groom (Fig. 1B). They also showed more paw licking behavior, rostral grooming and rostral transition frequency, as well as specific "no behavior to paw licking" and "paw licking to no behavior" transitions (Fig. 1B). While SERT$^{+/-}$ mice trended toward higher head wash frequency, "paw licking to head wash" transitions and "head wash to no behavior" transitions, we found no differences in automated data for correct vs. incorrect transitions, the percentage of caudal vs. rostral grooming episodes, or any other specific grooming transitions (Table 1). Finally, there were no significant differences in non-grooming behavior between SERT$^{+/-}$ mice and their wild-type littermates, including both manual endpoints (i.e., vertical rears and defecation boli) and automated endpoints (e.g., distance traveled, average velocity, turning angle and meandering; Fig. 1C).

Table 2
Additional manual and automated BDNF grooming and non-grooming endpoints. For manual analysis, zero values were seen in both groups for "no behavior to tail/genital wash", "paw licking to tail/genital wash", "head wash to tail/genital wash", "tail/genital wash to paw licking", "tail/genital wash to head wash" and "tail/genital wash" transitions. Transitions containing a "tail/genital wash" component were only characterized manually, due to the current difficulty of distinguishing this episode in the HomeCageScan software. All data is presented as mean ± SEM.

Endpoints	+/+	+/−
Manual analysis		
Head wash frequency	2.6 ± 0.8	0.9 ± 0.3[#]
Body/leg wash frequency	1.7 ± 0.7	0.3 ± 0.2[#]
Tail/genital wash frequency	0.2 ± 0.1	0 ± 0
Caudal episode frequency	0.2 ± 0.1	0.1 ± 0.1[#]
Caudal transition frequency	1.9 ± 1.2	0.3 ± 0.2
Relative % of caudal episodes	15 ± 6.0	9.0 ± 7.0
Relative % of rostral episodes	85 ± 6.0	91 ± 7.0
No behavior > body/leg wash	0.7 ± 0.6	0 ± 0
Head wash > body/leg wash	0.5 ± 0.3	0 ± 0[#]
Body/leg wash > no behavior	0.8 ± 0.6	0.3 ± 0.2
Body/leg wash > paw licking	0.4 ± 0.2	0 ± 0[#]
Body/leg wash > head wash	0.3 ± 0.2	0 ± 0
Body/leg wash > tail/genital wash	0.2 ± 0.1	0 ± 0
Tail/genital wash > no behavior	0.2 ± 0.1	0 ± 0
% correct transitions	49 ± 3.0	55 ± 5.0
Defecations	1.5 ± 0.4	1.4 ± 0.6
Automated analysis		
Head wash frequency	2.1 ± 0.6	0.8 ± 0.3[#]
Body/leg wash frequency	2.4 ± 0.7	0.5 ± 0.3[*]
Caudal episode frequency	2.4 ± 0.7	0.5 ± 0.3[*]
Caudal transition frequency	1.8 ± 0.6	0.5 ± 0.4[#]
Relative % of caudal episodes	27 ± 5.0	12 ± 6.0[#]
Relative % of rostral episodes	73 ± 5.0	88 ± 6.0[#]
No behavior > body/leg wash	0.6 ± 0.3	0.4 ± 0.3
Head wash > body/leg wash	0.6 ± 0.3	0.1 ± 0.1
Body/leg wash > no behavior	1.2 ± 0.4	0.1 ± 0.1
Body/leg wash > paw licking	0.9 ± 0.2	0.5 ± 0.3
Body/leg wash > head wash	0.2 ± 0.2	0 ± 0
% correct transitions	42 ± 5.0	28 ± 7.0

[*] $p < 0.05$ (trend); *U*-test vs. wild-type mice ($n = 9$ per group).
[#] $p = 0.05–0.01$ (trend); *U*-test vs. wild-type mice ($n = 9$ per group).

3.2. Grooming and non-grooming behavior of BDNF mutant mice

Analysis of manual data showed reduced grooming activity, including bouts, episodes, total duration and total transitions in BDNF$^{+/-}$ compared to BDNF$^{+/+}$ mice (Fig. 2A). We also observed decreased paw licking, rostral grooming, rostral transitions, and "no behavior to paw licking", "paw licking to no behavior", "paw licking to head wash" and "head wash to paw licking" transitions in mutant mice compared to controls (Fig. 2A). BDNF$^{+/-}$ mice tended to exhibit fewer head wash, body/leg wash and caudal grooming episodes, as well as "head wash to body/leg wash" and "body/leg wash to paw licking" transitions. Similar to SERT$^{+/-}$ mice, there were no genotype differences in total correct vs. incorrect transitions, percentage of rostral vs. caudal grooming episodes, or other specific grooming transitions in BDNF$^{+/-}$ mouse group (Table 2).

Applying behavior-recognition software to BDNF$^{+/-}$ and wild-type mice, we found global decreases in BDNF$^{+/-}$ mouse grooming bouts, episodes and total transitions, consistent with lower paw licking, body/leg wash, rostral grooming and caudal grooming frequency (Fig. 2B). For specific transitions, BDNF$^{+/-}$ mice exhibited fewer "paw licking to body/leg wash" and "body/leg wash to no behavior" transitions (Fig. 2B). They also displayed non-significant

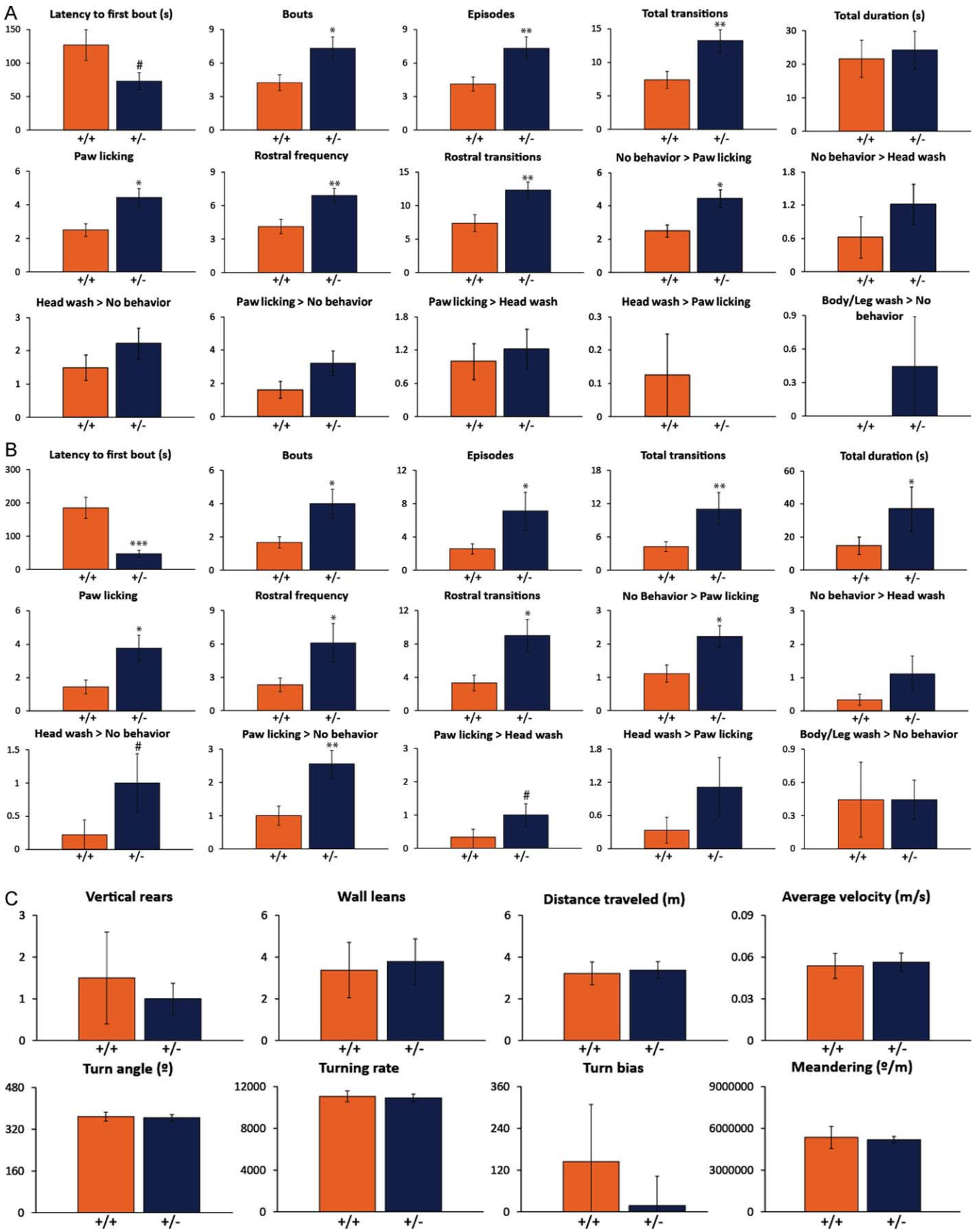

Fig. 2. Grooming and non-grooming behavior of BDNF mutant mice. (A) Manual analysis of grooming behavior. (B) Automated analysis of grooming behavior as performed by HomeCage Scan software (CleverSys, Inc.). (C) Analysis of non-grooming behavior performed by manual observers (vertical rears and wall leans) and EthoVision XT7 software (Noldus IT). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, $^{\#}p = 0.05–0.01$ (trend); *U*-test vs. wild-type mice ($n = 9$ per group).

trends toward lower head wash frequency, longer latency to groom, and higher relative percentage of rostral grooming episodes (Fig. 2B; Table 2). BDNF$^{+/-}$ mice showed a trend to fewer "no behavior to paw licking" transitions, "paw licking to head wash" transitions, frequency of rostral transitions and frequency of caudal transitions (Table 2). No differences were observed for total grooming duration, correct vs. incorrect transitions or other specific grooming transitions. As shown in Fig. 2C, there were no genotype differences for all manual non-grooming endpoints, including protected and unprotected vertical rears and defecation boli. In contrast, we found significantly reduced turning angle and turning rate in BDNF$^{+/-}$ mice, accompanied by a trend to lower distance traveled and average velocity, but not turning bias or meandering, compared to wild-type animals (Fig. 2C).

3.3. Correlational analysis of manual and automated data

Finally, grooming bouts, episodes, duration and total transitions data from SERT and BDNF cohorts were analyzed by the Spearman's rank correlation test. For SERT mice, significant correlations were found for total grooming duration ($R = 0.66$, $p < 0.005$), grooming bouts ($R = 0.62$, $p < 0.05$), grooming episodes ($R = 0.55$, $p < 0.05$) and total transitions ($R = 0.60$, $p < 0.05$). BDNF mice displayed similar significant correlations for total grooming duration ($R = 0.50$, $p < 0.05$), grooming bouts ($R = 0.50$, $p < 0.05$), grooming episodes ($R = 0.86$, $p < 0.0005$) and total transitions ($R = 0.84$, $p < 0.001$).

4. Discussion

SERT and BDNF are crucial for a wide array of molecular and behavioral mechanisms, and have long been studied in biobehavioral research. However, this is the first study examining in-depth alterations in grooming behavior and its syntax in SERT$^{+/-}$ and BDNF$^{+/-}$ mice. This focus is important because grooming represents a complex patterned behavior commonly seen in rodent and non-human primate models. Additionally, human psychiatric disorders involving stereotyped repetitive behaviors (e.g., autism, OCD and ADHD) have been associated with disruptions in SERT and BDNF genes (Bloch et al., 2008; Firk and Markus, 2009; Fontenelle et al., 2012; Maina et al., 2010; Nishimura et al., 2007; Sutcliffe et al., 2005).

Overall, SERT$^{+/-}$ mice exhibited profound increases in self-grooming behavior in response to novelty, although other (non-grooming) phenotypes remained unaltered (Fig. 1). In addition to global increases in grooming duration, bouts and episodes, SERT$^{+/-}$ mice displayed altered patterning (syntax) of their grooming, both initiating and aborting more grooming bouts (as reflected by increased "no behavior to paw licking" and "paw licking to no behavior" transitions). While this phenotype may be relevant to impaired impulse control (linked to serotonin homeostasis in both rodents (Ferrari et al., 2005) and humans (Pavlov et al., 2012)), another likely explanation is increased responsiveness to stress, previously reported in SERT$^{+/-}$ and SERT$^{-/-}$ mice (Li et al., 1999, 2000; Murphy et al., 2001). Although SERT mutant mice show lower basal levels of corticosterone, they respond to acute stressors with a heightened release of adrenocorticotropic hormone and oxytocin (Li et al., 1999, 2000; Murphy and Lesch, 2008; Murphy et al., 2001). Because exaggerated stress reactivity contributes to anxiety-related behaviors exhibited by SERT$^{+/-}$ and SERT$^{-/-}$ mice (Ansorge et al., 2004; Holmes et al., 2003a,c; Thakker et al., 2005; Zhao et al., 2006), the increased self-grooming observed here may be due to elevated anxiety-related responses (Kalueff et al., 2007a; Kalueff and Tuohimaa, 2004, 2005a,b). Furthermore, SERT mutant mice show significant changes in cortical morphology and development (Persico et al., 2001; Salichon et al., 2001), and the resulting disruption in sensory processing also may contribute to the alterations of self-grooming syntax seen in this study.

Although the constitutive knockout is the most common disruption of the SERT gene, recent reports have utilized other genetic manipulations for the investigation of the relationship between repetitive behaviors and SERT function. Integrin alphaIIbbeta3 regulates blood serotonin levels through an interaction with SERT (Carneiro et al., 2008; Weiss et al., 2004), and mice deficient for the integrin beta3 (Itgb3) gene show decreased self-grooming behavior (Carter et al., 2011). The substitution of alanine for glycine at codon 56 of the SERT gene (Gly56Ala) is a low-frequency genetic variant clinically associated with autism spectrum disorders and behavioral rigidity (Sutcliffe et al., 2005). Mice expressing this allele show increased plasma serotonin concentration, SERT activity and stereotyped hanging behavior (Veenstra-Vanderweele et al., 2012). Therefore, both abnormally high and abnormally low SERT activity may increase repetitive behavior, but the affected behavioral domains may be distinct. This further supports the pleiotropy of SERT dysfunction and the importance of serotonin homeostasis in the etiology of rigid and compulsive behaviors.

Interestingly, an earlier study using female SERT$^{-/-}$ and SERT$^{+/-}$ mice on a C57BL/6J background did not find altered grooming activity or patterning (Kalueff et al., 2007b), raising the possibility of sex differences in grooming behaviors in this mutant mouse strain. However, our mouse findings parallel observations in SERT$^{-/-}$ rats (Muller et al., 2010), which groom significantly more than SERT$^{+/+}$ rats. In line with this, SERT mutant mice on a C57BL/6J genetic background showed higher home-cage grooming frequency in SERT$^{-/-}$ vs. wild type mice (Lewejohann et al., 2010). While SERT$^{+/-}$ mice did not significantly differ from controls in this study, they showed an intermediate phenotype between high-grooming SERT$^{-/-}$ and low-grooming wild type controls. As already mentioned, mutations in the human SERT gene have been associated with disorders involving repetitive behaviors (Bloch et al., 2008; Murphy et al., 2004; Sutcliffe et al., 2005). Therefore, while future studies are needed to develop novel experimental models of SERT-related behavioral abnormalities, our data supports the connection between SERT activity and rigid behavioral patterning.

In contrast to SERT$^{+/-}$ mice, BDNF$^{+/-}$ mutants displayed a general decrease in self-grooming behavior (including lower duration, fewer transitions and bouts), as well as a trend toward reduced distance traveled (Fig. 2). *BDNF* mutations have been strongly associated with anxiety-related behaviors in mice (Chen et al., 2006) (also see Chourbaji et al., 2011 for discussion). Our findings do not directly implicate anxiety in the observed profile of BDNF$^{+/-}$ mice, since altered locomotion in the testing arena, previously reported in BDNF mutant mice (Dluzen et al., 2002; Einat et al., 2003), may itself account for the decrease in self-grooming. Because BDNF is a survival factor for motor neurons (Koliatsos et al., 1993; Martinowich and Lu, 2008), the degeneration in motor circuitry evoked by BDNF haploinsufficiency may contribute to the decrease in self-grooming behavior seen in BDNF$^{+/-}$ mice.

In humans, reduced serum levels of BDNF have been associated with OCD (Fontenelle et al., 2012; Maina et al., 2010), while elevated serum BDNF has been associated with autism (Nishimura et al., 2007) – both syndromes which involve rigid-compulsive behavior. Interestingly, lower levels of BDNF (particularly in the striatum) have been associated with decreased synaptic performance in dopamine neurons (Joyce et al., 2004; Laviola et al., 2004; Pineda et al., 2005), which prominently affect stereotyped behaviors, such as grooming. The loss of striatal dopaminergic tone in BDNF$^{+/-}$ mice is likely to contribute to the global decrease in locomotion and stereotyped behavior observed in this study. Moreover, our results are in line with previous data on increased grooming in BDNF-overexpressing mice (Papaleo et al., 2011) and mice injected with BDNF (Cirulli et al., 2004), thereby strongly supporting the

important role of BDNF in the bidirectional modulation of rodent self-grooming behavior.

Our early studies have shown that grooming in inbred mice can be reliably detected using automated behavior-recognition tools (Kyzar et al., 2011). The present study extends the application of automated high-throughput grooming analysis to genetically modified SERT$^{+/-}$ and BDNF$^{+/-}$ mice, demonstrating strong correlation of manual and automated grooming data in both high-grooming (SERT$^{+/-}$) and low-grooming (BDNF$^{+/-}$) mutant mouse strains. Because greater statistical significance was found through analysis of software-generated data, automated analysis may generally be better suited to the characterization of complex patterned behaviors such as self-grooming. However, while the observed values were not perfectly comparable across detection methods, both manual and automated methods detected relative changes in behavior between genotypes (Figs. 1 and 2). Future studies can utilize these approaches to characterize the behaviors of other mutant mouse strains, particularly those relevant to repetitive behavioral syndromes such as autism spectrum disorders, OCD and ADHD. For example, a growing number of strains with mutations in genes relevant to rigid-compulsive behavior, including the BTBR strain (Silverman et al., 2010), *Sapap3* (Welch et al., 2007), *Shank3* (Peca et al., 2011), *Cntnap2* (Penagarikano et al., 2011), oxytocin receptor (*Oxtr*) (Pobbe et al., 2012) and *Hoxb8* (Greer and Capecchi, 2002) mutants, show alterations in self-grooming behavior. In addition to genetic manipulations, the effect of various pharmacological agents on grooming behavior also may provide insights into potential treatments for repetitive behavioral syndromes (Mehta et al., 2011; Silverman et al., 2010).

Finally, there were several limitations in this study. For example, although not in the scope of this research, future studies may correlate gene and protein expression in specific brain regions (particularly the striatum, medulla and cerebellum) to alterations in grooming syntax. Additionally, mutant mice were tested here in a novel environment, and the results seen may not reflect self-grooming levels assessed in other contexts. As already mentioned, tail and genital grooming (albeit important for grooming analysis) was not quantified here due to the difficulty of distinguishing these behaviors from body/leg washes in the current HomeCageScan software. However, we expect that this aspect can easily be addressed once more sophisticated IT-based behavior-recognition technology becomes available.

In conclusion, SERT$^{+/-}$ and BDNF$^{+/-}$ knockout mice display distinct alterations in grooming behavior, suggesting that decreased SERT and BDNF expression modulate self-grooming (and its patterning) in opposite directions. Empowered by high-throughput automated behavior-recognition approaches, the comprehensive analysis of repetitive behaviors exhibited by these mutant mice has the potential to elucidate neural correlates of complex motor phenotypes, including modeling human brain disorders already clinically linked to genetic differences in SERT and BDNF signaling pathways.

Conflict of interest statement

The authors have no conflict of interest regarding this study.

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