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Daniela Babovic
Royal College of Surgeons in Ireland

Colm M. O'Tuathaigh
Royal College of Surgeons in Ireland

Gerard J. O'Sullivan
Royal College of Surgeons in Ireland

Jeremiah J. Clifford
Royal College of Surgeons in Ireland

Orna Tighe
Royal College of Surgeons in Ireland

See next page for additional authors

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Authors
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Exploratory and habituation phenotype of heterozygous and homozygous COMT knockout mice

Daniela Babovic[a], Colm M. O'Tuathaigh[a], Gerard J. O'Sullivan[a], Jeremiah J. Clifford[a], Orna Tighe[a], David T. Croke[a], Maria Karayiorgou[b], Joseph A. Gogos[b], David Cotter[c] and John L. Waddington[a*]

[a] Molecular & Cellular Therapeutics and [c] Department of Psychiatry, Royal College of Surgeons in Ireland, Dublin, Ireland and [b] Columbia University, New York, USA

[*]Corresponding author at: Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, St Stephen's Green, Dublin 2, Ireland

Tel: +353 1 402 2129

Fax: +353 1 402 2453

Email: jwadding@rcsi.ie

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Abstract

Catechol-*O*-methyltransferase (COMT) inactives dopamine in prefrontal cortex and is associated clinically with a schizophrenia endophenotype. Using an ethologically based approach, the phenotype of mice with heterozygous COMT deletion was characterised by decreased rearing with increased sifting and chewing. Heterozygous COMT deletion is associated with a distinctive phenotype. This differs from that which we have reported previously for heterozygous deletion of the schizophrenia risk gene neuregulin-1.

Keywords: COMT, knockout mice, behavioural phenotype, ethogram, exploration, habituation, neuregulin, schizophrenia

Several genes have now been associated with risk for schizophrenia [9,12,15,25]. However, the functional significance of these gene associations is poorly understood. The genes implicated are diverse and their individual effect sizes are small. Each gene might contribute to overall risk for diagnosis of schizophrenia, perhaps via expression in convergent pathways. Alternatively, specific genes might influence risk for distinct aspects [endophenotypes] of the overall schizophrenia syndrome.

Neuregulin-1 (NRG1) and catechol-*O*-methyltransferase (COMT) are two genes that have attracted particular attention. While NRG1 is one of the most established risk genes for schizophrenia in association studies, its functional role is unclear [3,13,19]. Conversely, while evidence for association between COMT and risk for schizophrenia is inconsistent [5,18], it may be associated functionally with a schizophrenia endophenotype. In prefrontal cortex (PFC), the dopamine (DA) catabolising enzyme COMT is critically involved in the inactivation of DA. Thus, low [Met/Met], intermediate [Val/Met] and high [Val/Val] activity COMT $Val^{158/108}$ Met genotypes are associated with decreasing performance in tests of executive function and working memory that are thought to involve dopaminergic mechanisms in PFC [11,27].

The functional roles of genes such as NRG1 and COMT can be investigated through the phenotype of mice with targeted gene deletion [knockout; 1,2,22]. Phenotypic studies in COMT knockouts have involved behavioural paradigms which include general motor activity, anxiety, aggression and sensorimotor gating [8,10,14]. Additionally, low COMT activity may fail to buffer the effect of related 22q11 mutations that also effect DA turnover in the PFC [26]. The value of an ethologically based approach to behavioural characterisation of mutant mice is increasingly recognised. It can identify novel

phenotypic effects and resolve apparent inconsistencies in phenotype [4,7,22,23]. We have developed and applied such an approach to mice with knockout of each of the five DA receptor subtypes and components mediating DA receptor signal transduction [28]. This approach quantifies the ethogram of how all the individual topographies of behaviour in the mouse repertoire change and interchange as the animal interacts with its environment over time, from initial exploration, through habituation to quiescence. The ethogram is not a specific model of psychosis. Rather, it measures the basic processes of environmental interaction and adaptation that are among those disrupted in psychotic illness. Such fundamental information is essential before proceeding to more specific behavioural models relating to schizophrenia.

Having applied this approach to NRG1 mutants [22,23], in the present study we examine in the same manner the functional role of the COMT gene. This allows us to address two critical questions. Firstly, what is the phenotype of COMT mutants at this fundamental level of behaviour? Secondly, is this phenotype similar to or different from that which we have reported for NRG1 mutants using identical methods?

Mice containing the mutated COMT allele were generated at Rockefeller University, New York, USA, as described previously [8]. To establish a congenic line, mice of this hybrid [129/J × C57BL6] strain with heterozygous deletion of the COMT gene were backcrossed to wildtype C57BL6 mice for ten generations. Mutants and wildtypes of this tenth generation were then transported to Dublin. Here, analysis of tail DNA by polymerase chain reaction was used to identify wildtypes (WT: $COMT^{+/+}$) and mice with heterozygous (HET: $COMT^{+/-}$) and homozygous (KO: $COMT^{-/-}$) deletion of the COMT gene among the offspring of HET breeding pairs. Mice were housed in groups of 3-5 per

cage and maintained at 21±1°C on a 12:12 h light-dark cycle (08:00 h on; 20:00 h off], with *ad libitum* access to food and water. Experimental animals were from litters of the same generational age. These studies were approved by the Research Ethics Committee of the Royal College of Surgeons in Ireland and were conducted under license from the Department of Health and Children in accordance with Irish legislation and the European Communities Council Directive 86/609/EEC for the care and use of experimental animals.

For evaluation of the ethogram of COMT mutants, experimentally naïve mice were removed from their home cages and placed individually in clear glass observation chambers (36 × 20 × 20 cm) under standard room conditions (light 150 Lux). Behavioural assessments were carried out using a rapid time-sampling behavioural checklist technique, as described previously in detail [20,21,23,28]. In outline, 10 mice were observed individually for 5 s periods at 1 min intervals over 15 consecutive minutes, using an ethologically based behavioural checklist. This technique enables the observer to determine the presence or absence of the following individual topographies of behaviour in each 5 s sample period: sniffing, locomotion, total rearing [of any form], rearing seated, rearing free, rearing to wall, sifting, chewing, total grooming [of any form] and intense, syntactic grooming. This cycle of assessment by behavioural checklist over an initial 15 min period was repeated twice over an initial exploratory period of 60 min. Evaluation was then continued across 8 x 10 min cycles, from 80-90 through to 360–370 min. For each animal, behaviour was evaluated once only by an observer who was blind to genotype.

For the ethogram of initial exploration, total counts for each behaviour, i.e. the number of 5 s observation periods in which a given behaviour is manifested, were calculated for each mouse across the first three 15 min periods. For the ethogram of subsequent habituation, total counts for each behaviour were summed similarly over eleven 10 min periods. Data were expressed as means ± S.E.M. and subjected to square root transformation to facilitate ANOVA, as described previously [20,21,23]. Topographies of behaviour over initial exploration were analysed using three-way ANOVA with main factors of genotype, sex and age. Topographies of behaviour over subsequent habituation were analysed using repeated measures ANOVA with these same factors evaluated across time bins. Where appropriate, *post-hoc* comparisons were carried out using independent tests for multiple comparisons. All statistical analyses were carried out using the SPSS software package [Version 14, SPSS Inc.].

This study involved 120 mice [20 male and 20 female for each of WT, HET and KO genotypes; age range 64 – 146 days]. Mean body weight did not differ between KO (male, 24.5 ± 0.4 g; female, 18.6 ± 0.4 g), HET (male, 23.9 ± 0.4 g; female, 18.3 ± 0.4 g) and WT (male, 24.1 ± 0.4 g; female, 19.3 ± 0.3 g) mice. On qualitative inspection of posture, reactivity to handling and general activity, no gross motor phenotype was apparent.

Over initial exploration, total rearing [effect of genotype, $F(2,116)=3.77$, $p < 0.05$], particularly rearing free [effect of genotype, $F(2,116)=5.67$, $p < 0.005$], differed over the 60-min period of assessment in the absence of any genotype × sex interactions; this derived essentially from reduced levels of rearing free in HET mutants [males: HET *vs.* KO $p < 0.05$; females: HET *vs.* KO $p < 0.01$, HET *vs.* WT $p < 0.05$] (Fig. 1). Sifting

[effect of genotype, $F(2,116)=3.98$, $p < 0.05$] and chewing [effect of genotype, $F(2,116)=7.83$, $p < 0.001$] also differed in the absence of any genotype × sex interactions; this derived essentially from HET mutants evidencing increased levels of both sifting [males: HET *vs.* KO $p < 0.05$, HET *vs.* WT $p < 0.01$; females: HET *vs.* WT $p < 0.05$] and chewing [males: HET *vs.* KO $p < 0.01$; females: HET *vs.* KO $p < 0.05$, HET *vs.* WT $p < 0.05$]. There were no effects of genotype for locomotion, sniffing, rearing to wall and total grooming; levels of rearing seated were too low to be recorded, while levels of intense grooming were too low for meaningful analysis. As expected, individual topographies of behaviour varied with sex and age. However, there were no interactions with genotype.

Over subsequent habituation, rearing free initially increased before subsequently declining readily over the 370 min period of assessment [effect of time, $F(1,10)=4.03$, $p < 0.001$]; lower levels of rearing free in HET mutants during initial exploration persisted across time-bins [effect of genotype, $F(2,116)=3.50$, $p < 0.05$; for both males and females: HET *vs.* KO $p < 0.05$, HET *vs.* WT $p < 0.05$ across time bins] (Fig. 2). Sifting declined readily [effect of time, $F(1,10)=17.04$, $p < 0.001$]; higher levels of sifting in HET mutants during initial exploration persisted across time-bins [effect of genotype, $F(2,116)=3.19$, $p < 0.05$; for both males and females: HET *vs.* KO $p < 0.05$, HET *vs.* WT $p < 0.05$ across time bins]. Levels of chewing across time-bins were too low for meaningful analysis. As expected, other topographies of behaviour varied over time and with sex and age. However, there were no interactions with genotype.

The exploratory phenotype of COMT deletion was not characterized by any generic change in ‘activity’, as noted previously [8,10,14]. Rather, the present behavioural

approach revealed reduction in rearing free and increases in sifting and chewing. This phenotypic effect was evident in HET rather than KO mutants and a similar profile has been reported previously for increased aggression [8]. For some processes, HET mutation may reduce COMT activity only to a level above a threshold for induction of compensatory mechanisms. Conversely, full KO may reduce COMT below this threshold to result in induction of compensatory mechanisms that are able to sustain functions otherwise mediated by COMT [8]. Also, differences in compensation in relation to gene dosage may differ between cellular and behavioural levels of function. These issues of neuronal compensation have been the subject of recent reviews [17,28]. In contrast to certain other behaviours [8], there were no sex-specific phenotypic effects at the level of exploration. Expected patterns of change in exploratory behaviour with age did not differ between the genotypes, indicating that COMT mutation does not interact with maturation in the regulation of exploration over this period of young adulthood. However, the age range studied was limited and future studies should examine these relationships over a wider age range that includes sexual maturation. This exploratory phenotype of COMT mutants differed from that of NRG1 mutant counterparts, which evidenced increased locomotion and rearing free, with sex-specific alterations in sifting and grooming, when assessed using identical methods [22,23].

These reductions in rearing free and increases in sifting in COMT mutants over initial exploration continued into the habituation period. Expected patterns of change in habituation with age did not differ between the genotypes, indicating that COMT mutation does not interact with maturation in the regulation of habituation processes over this limited period of young adulthood. This habituation phenotype of COMT mutants

differed from that of NRG1 mutant counterparts, which evidenced enduring increases in locomotion and rearing free, emergent decreases in sniffing and rearing and sex-specific alterations in sifting and grooming [22,23].

Though exploration is a multifaceted behaviour [16], exploration and subsequent habituation are dependent on memory for a familiar environment together with associated processes in PFC [6]. COMT is an important regulator of aspects of DA-dependent working memory in PFC that are known to be disturbed in schizophrenia [11,27]. Thus, as there is no overall increase in general activity in COMT mutants, disruption of exploration and habituation may involve a schizophrenia-related disruption of DA-dependent working memory. Conversely, NRG1 is involved in distinct processes, including CNS development, synapse formation, neuronal migration and synaptic plasticity, that are thought to be dysfunctional in schizophrenia [3,13]. It is increasingly recognised that schizophrenia is influenced by several genes of small effect, including COMT and NRG1. However, none are clearly associated with a specific schizophrenia endophenotype [5,9,12,13,18,19,27].

In summary, mutants such as those studied here constitute models of the functional roles of genes implicated in schizophrenia [1,2,22]. They indicate that heterozygous deletion of COMT is associated with a characteristic exploratory and habituation phenotype. Furthermore, this phenotype is distinct in several important aspects from that associated with heterozygous deletion of NRG1. COMT mutation and NRG1 mutation each disrupt fundamental processes of initial exploration of and subsequent habituation to the environment, but with differing behavioural, temporal and sex specificities. Importantly, these data indicate that within this overall domain of function, COMT and

NRG1 appear to act through separate rather than convergent mechanisms and thus may influence distinct aspects of the overall schizophrenia syndrome. These findings constitute a basis for investigating the relative roles of COMT and NRG1 in models of the core cognitive and social behaviours that are impaired in schizophrenia.

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Fig. 1. Phenotypic ethogram of behavioural topography over an initial 60 min period of exploration. Data are mean behavioural counts ± S.E.M. for locomotion, total rearing, rearing free, sniffing, rearing to wall, grooming, sifting and chewing in wildtype (WT) male (black columns; n = 20) and female (grey columns with centre black; n = 20), COMT heterozygous (HET) male (grey columns; n = 20) and female (grey hatched columns; n = 20) and COMT homozygous knockout (KO) male (open columns; n = 20) and female (open hatched columns; n = 20) mice. * $p < 0.05$, ** $p < 0.01$ HET *vs.* KO or HET *vs.* WT as indicated.

Fig. 2. Phenotypic ethogram of behavioural topography over a 370 min period of habituation. Data are mean behavioural counts ± S.E.M. for n = 20 per group per 10 min period for locomotion, rearing free and sifting in wildtype (WT, black squares), COMT heterozygous (HET, black triangles) and COMT homozygous (KO, open triangles) knockouts for (a) male and (b) female mice. [a] $p < 0.05$ HET *vs.* KO, [b] $p < 0.05$ HET *vs.* WT.

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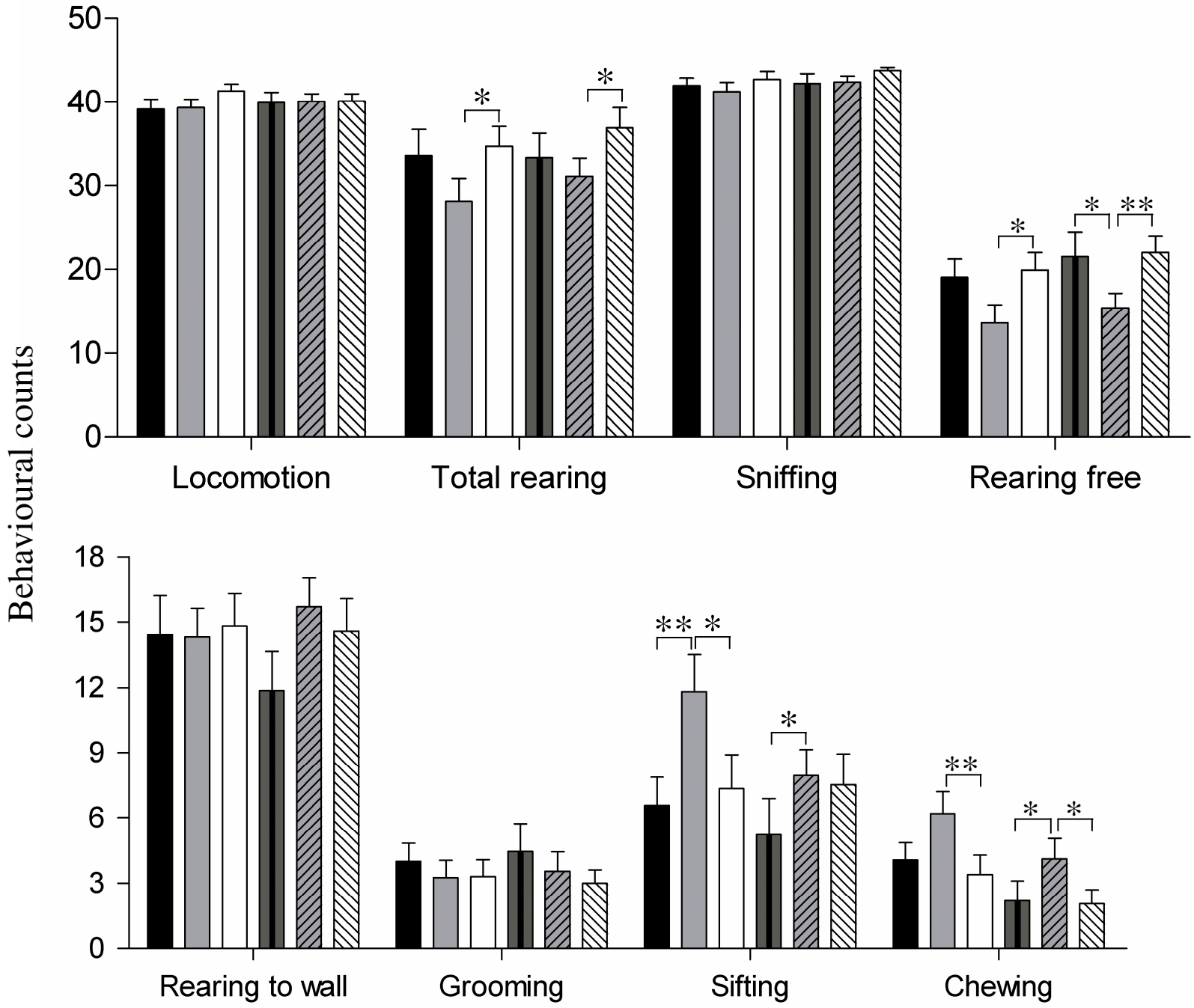
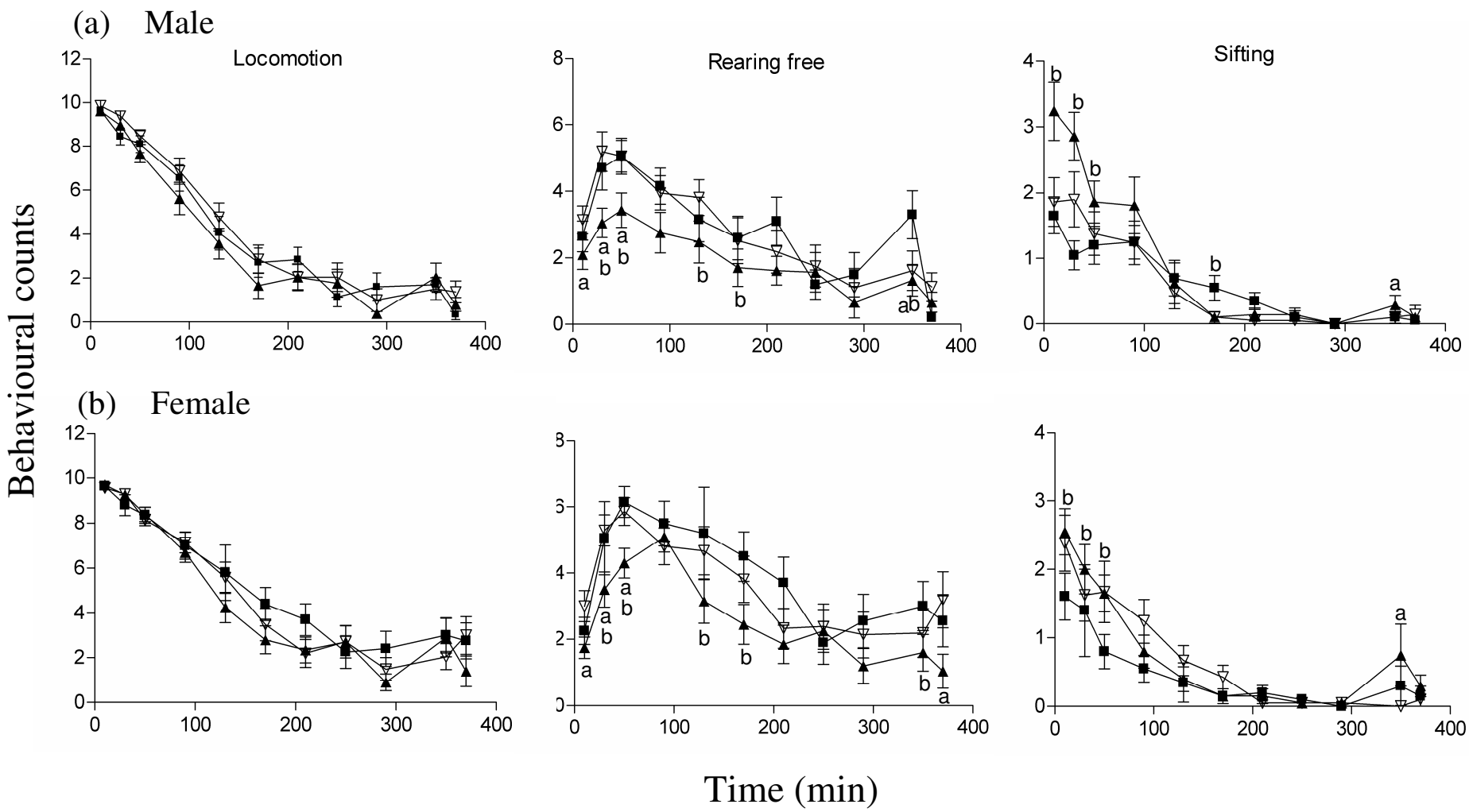

Behavioural counts
50
40
30
20
10
0
Locomotion
Total rearing
Sniffing
Rearing free
18
15
12
9
6
3
0
Rearing to wall
Grooming
Sifting
Chewing


(a) Male
Locomotion
Rearing free
Sifting
(b) Female
Behavioural counts
Time (min)