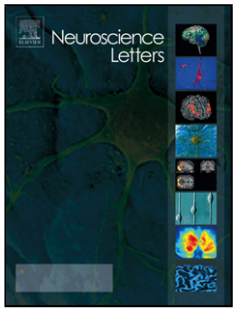
CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Semaphorin 6A knockout mice display abnormalities across ethologically-based topographies of exploration and in motor learning
Authors	Håkansson, Kerstin;Runker, Annette;O'Sullivan, Gerard J.;Mitchell, Kevin J.;Waddington, John L.;O'Tuathaigh, Colm M. P.
Publication date	2017-01-18
Original Citation	Håkansson, K., Runker, A., O'Sullivan, G. J., Mitchell, K. J., Waddington, J. L. and O'Tuathaigh, C. M. P. (2017) 'Semaphorin 6A knockout mice display abnormalities across ethologically-based topographies of exploration and in motor learning', Neuroscience Letters, 641, pp. 70-76. doi:10.1016/j.neulet.2017.01.043
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.neulet.2017.01.043
Rights	© 2017 Elsevier B.V. All rights reserved. This manuscript version is made available under the CC-BY-NC-ND 4.0 license. - http://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2024-03-29 10:47:09
Item downloaded from	https://hdl.handle.net/10468/4458

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Accepted Manuscript



Title: Semaphorin 6A knockout mice display abnormalities across ethologically-based topographies of exploration and in motor learning

Authors: Kerstin Håkansson, Annette E. Runker, Gerard J. O'sullivan, Kevin J. Mitchell, John L. Waddington, Colm M.P. O'Tuathaigh

PII: S0304-3940(17)30064-2
DOI: http://dx.doi.org/doi:10.1016/j.neulet.2017.01.043
Reference: NSL 32585

To appear in: *Neuroscience Letters*

Received date: 16-11-2016
Revised date: 11-1-2017
Accepted date: 17-1-2017

Please cite this article as: Kerstin Håkansson, Annette E.Runker, Gerard J.O'sullivan, Kevin J.Mitchell, John L.Waddington, Colm M.P.O'Tuathaigh, Semaphorin 6A knockout mice display abnormalities across ethologically-based topographies of exploration and in motor learning, Neuroscience Letters http://dx.doi.org/10.1016/j.neulet.2017.01.043

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Semaphorin 6A knockout mice display abnormalities across ethologically-based topographies of exploration and in motor learning

Kerstin Håkansson [a], Annette E. Runker [b], Gerard J. O'Sullivan [a], Kevin J. Mitchell [b], John L. Waddington [a], Colm M.P. O'Tuathaigh [a, c*]

[a] Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland

[b] Smurfit Institute of Genetics and Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland

[c] School of Medicine, University College Cork, Cork, Ireland

* Corresponding author at: School of Medicine, University College Cork, Cork, Ireland.

Tel: +353 211 490 5303

E-mail address: c.otuathaigh@ucc.ie

ACCEPTED MANUSCRIPT

Highlights

- Genetic disruption of the axon guidance gene, semaphorin-6A, produces abnormal exploratory behaviours in a novel environment in mice
- Semaphorin-6A mutant mice show disturbed habituation of exploration in a novel environment
- Heterozygous semaphorin-6A mutants exhibit disruption of motor learning.

Abstract

Semaphorins are secreted or membrane-bound proteins implicated in neurodevelopmental processes of axon guidance and cell migration. Exploratory behaviour and motor learning was examined ethologically in Semaphorin 6A (Sema6A) mutant mice. The *ethogram* of initial exploration in Sema6A knockout mice was characterised by increased rearing to wall with decreased sifting; over subsequent habituation, locomotion, sniffing and rearing to wall were increased, with reduced habituation of rearing seated. Rotarod analysis indicated delayed motor learning in Sema6A heterozygous mutants. Disruption to the axonal guidance and cell migration processes regulated by Sema6A is associated with topographically specific disruption to fundamental aspects of behaviour, namely the *ethogram* of initial exploration and subsequent habituation to the environment, and motor learning.

Keywords: Semaphorin 6A; Knockout mice; Behavioural phenotype; Ethogram; Motor Learning

ACCEPTED MANUSCRIPT

1. Introduction

Identification of genes affecting neural development and/or synaptic connectivity has been suggested to represent a promising approach to yield candidates for genetic studies of psychiatric disorders in humans [1, 2]. For example, this approach follows logically from evidence that mice mutant for genes associated with risk for psychotic illness show defects in neurodevelopment, such as alterations in brain morphology and connectivity, together with physiological and behavioural abnormalities [3-6].

Semaphorin 6A (Sema6A) is a member of the semaphorin family of genes involved in cell migration, axon guidance and synaptogenesis. Mutation of Sema6A in mice is associated with a spectrum of subtle defects in cell migration and axon guidance in various brain areas, including the thalamocortical system, hippocampus, cerebellum and various other structures [4, 7-12]. A recent gene expression analysis revealed alterations in semaphorin and plexin expression in the prefrontal cortex of patients with schizophrenia [13]. While phenotypic studies have identified cognitive and social behavior phenotypes reminiscent of schizophrenia in Sema6A mutants [4], the phenotype of Sema6A mutants at more fundamental levels of behaviour has yet to receive systematic investigation. The value of an ethologically based approach to behavioural characterisation of mutant mice, which takes into account species-specific characteristics, is illustrated in its ability to identify novel phenotypic effects and resolve apparent inconsistencies in phenotype [6, 14, 15]. We have developed and applied such an approach to mice with mutation of several genes associated with schizophrenia, including the neurodevelopmental genes neuregulin-1 [NRG1; 16] and disrupted-in-schizophrenia-1 [DISC-1; 17], and the pathophysiologically implicated gene catechol-*O*-methyl-transferase [COMT; 18]. This approach involves

ACCEPTED MANUSCRIPT

quantification of individual topographies of both externally- and internally-directed exploratory behaviour in the mouse repertoire and their interplay over an extended time frame, from initial exploration, through habituation to quiescence, i.e. the *ethogram* [15]. Motor learning, commonly accessed using the rotarod test, constitutes another fundamental level of behaviour.

In the present study we examine the functional role of the Sema6A gene in terms of the phenotype of Sema6A mutants at fundamental levels of behaviour, as a necessary complement to phenotypic studies at the level of cell migration, axon guidance and synaptogenesis. Additionally, these studies examine the extent to which this phenotype might be similar to or different from that which we have reported, using identical methods, in mice mutant for neurodevelopmental genes related to psychotic illness.

2. Methods

2.1 Animals

Mice containing the Sema6A mutation were generated at University of California, San Francisco, as described previously [7]. Analysis of tail DNA by polymerase chain reaction was used to identify wildtype (WT), heterozygous (HET) and homozygous knockout (KO) mutants among the offspring of heterozygous breeding pairs. Mice were housed in groups of 3-5 per cage and maintained at 21±1^{o}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

ACCEPTED MANUSCRIPT

Children in accordance with Irish legislation and the European Communities Council Directive 86/609/EEC for the care and use of experimental animals.

2.2 Behavioural assessments

For evaluation of the *ethogram* of Sema6A mutants, mice were removed from their home cages and placed individually in clear glass observation chambers (36 × 20 × 20 cm). Behavioural assessments were carried out using a rapid time-sampling behavioural checklist technique, as described previously in detail [16-19]. For this procedure, 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 behaviours (occurring alone or in any combination) in each 5 s sample period: locomotion (coordinated movement of all four limbs resulting in a change of location), sniffing (flaring of nostrils with movements of vibrissae), total rearing (rearing of any form); rearing seated (front paws reaching upwards with hind limbs on floor in sitting position), rearing free (front paws reaching upwards away from a cage wall while standing on hind limbs), rearing to wall (front paws reaching upwards onto or towards a cage wall while standing on hind limbs), sifting (characteristic sifting movements of the front paws through bedding material on cage floor), grooming (of any form), intense grooming (syntactic grooming of the snout and then face with the forepaws, followed by vigorous grooming of the hind flank or anogenital region with the snout), chewing (chewing movements directed onto physical material, i.e. cage bedding and/or faecal pellets, without consumption) and stillness (asleep or motionless with no behaviour evident). This cycle of assessment by behavioural checklist over a 15 min period (0-15 min) was repeated twice (20-35 and 40-55 min)

ACCEPTED MANUSCRIPT

over an initial exploratory period of 60 min. Continued evaluation using the checklist was then carried out across 8 × 10 min cycles, at 80–90, 120–130, 160–170, 200–210, 240–250, 280– 290, 340–350 and 360–370 min. For each animal, behaviour was evaluated once only by an observer who was blind to genotype.

Construction of the *ethogram* for each mouse across the initial exploratory phase (0–55 min) involved calculating total counts for each individual behaviour in terms of the number of 5 s observation periods in which a given behaviour is manifested, across the first three 15-min (0–15, 20–35, 40–55 min) cycle periods. These data were expressed as means ± SEM. Data for each topography of behaviour were analysed using analysis of variance (ANOVA) following square-root transformation. To determine the habituation profiles of these ethograms over prolonged observation, total counts for each individual behaviour were summed as above over each of the following time periods: 0–10, 20–30, 40–50, 80–90, 120–130, 160–170, 200–210, 240–250, 280–290, 340–350 and 360–370 min. These data were also expressed as means ± SEM and analysed using repeated measures ANOVA following square-root transformation [16, 18].

All mice were also tested on an accelerating rotarod (Panlab s.l., Barcelona, Spain) three weeks prior to the *ethogram*. Prior to commencing the experiment, three familiarisation trials were administered, each consisting of placement on the rotarod apparatus at a constant speed (4 rpm) until the mouse remained on the rotating rod for a continuous period of 60 s; each familiarisation trial was separated by an interval of at least 15 min. Training commenced 30 min after the final familiarisation trial. During each training session, the rotarod accelerated from 4 to 40 rpm over 5 min.

ACCEPTED MANUSCRIPT

Time until the mouse fell off the drum onto cushioning material was recorded across 6 consecutive training days of 4 sessions per day, with an inter-session interval of at least 30 min. The mean value for each animal across a given training day was used for statistical analysis. These data were expressed as means ± SEM and analysed using repeated measures ANOVA following square-root transformation.

3. Results

3.1 The *ethogram*: exploration during initial 60-min period

This study involved 60 mice [10 male and 10 female for each of WT, HET and KO genotypes; mean age 154 ± 33 days]; neither mean age nor body weight differed between the genotypes [$P > 0.05$]. On qualitative inspection of posture, reactivity to handling and general activity, no gross motor phenotype was apparent.

Over initial exploration, rearing to wall [effect of genotype, $F(2,52) = 3.57$, $P < 0.05$; no] and sifting [effect of genotype, $F(2,52) = 4.88$, $P < 0.05$], differed in the absence of any genotype × sex interactions; these effects of genotype derived primarily from increased rearing to wall and decreased sifting in KO mutants (Fig.1). There were no effects of genotype for locomotion, sniffing, total rearing, rearing free, rearing seated, total grooming and chewing; levels of intense grooming were too low for meaningful analysis (data not shown).

Independent of genotype, over exploration female mice exhibited higher levels of locomotion [effect of sex, $F(1, 52) = 5.21$, $P < 0.05$] and sniffing [effect of sex, $F(1, 52) = 3.75$, $P < 0.05$] and lower levels of grooming [effect of sex, $F(1, 52) = 5.37$, $P < 0.05$] relative to male mice.

ACCEPTED MANUSCRIPT

3.2 The *ethogram*: exploration during entire habituation period

Over subsequent habituation, each of locomotion [effect of time, $F(10,520) = 9.70$, $P < 0.001$], sniffing [effect of time, $F(10,520) = 9.75$, $P < 0.001$], total rearing [effect of time, $F(10,520) = 6.18$, $P < 0.001$], rearing to wall [effect of time, $F(1,10) = 16.15$, $P < 0.001$] and sifting [effect of time, $F(10,520) = 5.93$, $P < 0.001$] declined across time bins, in a manner that did not differ between the genotypes or between the sexes [no time × genotype or time × genotype × sex interactions]; low levels of rearing seated increased initially before declining subsequently, with this early increase being reduced and subsequent decline attenuated in KO mutants [time × genotype interaction, $F(20, 520) = 1.67$, $P < 0.05$]; low levels of rearing free declined over habituation in a manner that was disrupted in female KO mutants [time × genotype × sex interaction, $F(20, 520) = 1.61$, $P < 0.05$] (data not shown); total grooming did not vary systematically with time, while levels of chewing across time bins were too low for meaningful analysis (data not shown). Across habituation, overall levels of locomotion [effect of genotype, $F(2, 52) = 3.12$, $P < 0.05$], sniffing [effect of genotype, $F(2, 52) = 3.25$, $P < 0.05$], total rearing [effect of genotype, $F(2, 52) = 3.75$, $P < 0.05$] and rearing to wall [effect of genotype, $F(2, 52) = 5.42$, $P < 0.01$] differed between the genotypes; these effects of genotype derived primarily from increased levels in KO mutants (Fig. 2).

Independent of genotype, rates of habituation differed between male and female mice for locomotion [time × sex interaction, $F(10,520) = 1.95$, $P < 0.05$], sniffing [time × sex interaction, $F(10,520) = 2.93$, $P < 0.005$], chewing [time × sex interaction, $F(10,520) = 3.16$, $P < 0.005$] and stillness [time × sex interaction, $F(10,520) = 2.43$, $P<0.01$] (data not shown). Across habituation, female mice exhibited higher overall

ACCEPTED MANUSCRIPT

levels of locomotion [effect of sex, $F(1,52) = 7.01$, $P < 0.05$], sniffing [effect of sex, $F(1,52) = 5.85$, $P < 0.05$], total rearing [effect of sex, $F(1,52) = 4.61$, $P < 0.05$], rearing to wall [effect of sex, $F(1,52) = 4.78$, $P < 0.05$], and total grooming [effect of sex, $F(1,52) = 8.74$, $P < 0.005$] relative to male mice (data not shown).

3.3 Rotarod learning

While no overall effect of genotype on rotarod performance was observed across the six days of training, HET mutants exhibited a slower rate of improvement in rotarod performance across training days relative to WT and KO mutants [genotype x training day interaction, $F(10, 270) = 2.01$, $P < 0.05$; no genotype × training day × sex interaction] (Fig. 3). Independent of genotype, female mice exhibited higher overall performance on the rotarod relative to male mice [effect of sex, $F(1,54) = 8.36$, $P < 0.01$].

4. Discussion

The initial exploratory phenotype of Sema6A mutants, apparent primarily in KO with only limited evidence for intermediate, gene dosage effects in HET, was characterised by increased rearing to wall with increased sifting; this indicates a shift in exploratory behaviours from downwards, in the immediate locality of the mouse, to upwards, towards the perimeter of the environment and beyond. Over subsequent habituation, this increase in rearing to wall was sustained and accompanied by increased sniffing, which was distributed over a wider area via increased locomotion; however, only for low levels of rearing seated and rearing free was the core process of habituation [i.e. rate of change in a given behaviour with time] subtly disrupted, with disruption to rearing free being manifested in a sex-specific manner. Thus, Sema6A KO is

ACCEPTED MANUSCRIPT

associated specifically with an upward and outward shift in the topography of exploratory behaviours that broadens over time, with disruption to habituation confined to elements of rearing manifested more locally.

The motor phenotype of Sema6A mutants, apparent primarily in HET with only limited evidence for intermediate, gene dosage effects in KO, was characterised by impaired acquisition of rotarod performance; this is consistent with previous phenotypic data in Sema6A KO mutants indicating subtle abnormalities in gait and smooth motor action, in association with disruption to corticospinal circuitry [11, 20]. That this motor learning deficit was more evident in Sema6A HET than in KO may reflect HET mutation reducing Sema6A activity only to a level above a threshold for inducing compensatory mechanisms, with KO mutation reducing Sema6A to a lower level inducing compensatory mechanisms; that such processes may explain unexpected phenotypic differences in HET *vs* KO across distinct domains of function has been offered previously for COMT mutants [18, 21]. Additionally, specific migratory defects in Sema6A KO are variable and not highly penetrant; this may explain incomplete penetrance in some motor phenotypes and the presence of phenotypic effects in HET but not KO [4, 10, 11]. In agreement with the present study results, sex-specific effects among individual topographies of exploratory behaviour have also been reported in mice mutant for the NRG1 gene [16], as well as dopamine [DA] receptor subtype and related transduction mutants [15, 18, 19]. This may reflect an effect of sex (or sex hormones) on semaphorin/plexin signalling [22].

Recent neuroimaging, neurological and neuropathological studies in schizophrenia have indicated dysconnectivity in limbic, intracortical and particularly thalamocortical

ACCEPTED MANUSCRIPT

tracts that are a critical component of putative dysfunction in a fronto-striato-pallido-thalamo-cortical network [23-26]. These clinical findings parallel those in Sema6A mutants surprisingly closely [10], with such mutant findings extending to the cerebellum and corticospinal tract [8, 10, 11]; both of these regions have also been implicated in the pathobiology of schizophrenia [27-29]. Detailed analysis of the *ethogram*, as described here, indicates in Sema6A KO a hyperactive exploratory phenotype, implicating dysfunction in both motivational and motoric processes. The data from the *ethogram* is in agreement with observed hyperactive phenotypes reported for the Sema6A KO mutant using automated measures [4]. Such hyperactivity in response to stress, novelty or psychotomimetic agents has been considered a behavioural index of positive psychotic symptoms [30, 31], with a similar phenotype observed in several putative preclinical models of schizophrenia, including mice mutant for the neurodevelopmental risk genes NRG1 and DISC-1 [6, 16, 32]. Thus, the present data elaborate a growing body of evidence that mutations in Sema6A, and/or possibly interacting genes, may result in dysfunction at the level of neuronal networks with associated behavioural phenotypes of relevance to neuropsychiatric disorders.

5. Acknowledgements

These studies were supported by a Science Foundation Ireland Principal Investigator grant (07/IN.1/B960) a Postdoctoral Fellowship from the Health Research Board (PD/2007/20) and a Postdoctoral Fellowship from the Irish Research Council for Science, Engineering and Technology.

ACCEPTED MANUSCRIPT

6. References

[1] H. Jaaro-Peled, Y. Ayhan, M.V. Pletnikov, A. Sawa, Review of pathological hallmarks of schizophrenia: comparison of genetic models with patients and nongenetic models, Schizophr. Bull. 36 (2010) 301-13.

[2] S. Horváth, K. Mirnics, Schizophrenia as a disorder of molecular pathways, Biol. Psychiatry. 77 (2015) 22-8.

[3] P.A. Arguello, J.A. Gogos, Modeling madness in mice: one piece at a time, Neuron. 52 (2006) 179-96.

[4] A.E. Rünker, C. O'Tuathaigh, M. Dunleavy, D.W. Morris, G.E. Little, A.P. Corvin, M. Gill, D.C. Henshall, J.L. Waddington, K.J. Mitchell, Mutation of Semaphorin-6A disrupts limbic and cortical connectivity and models neurodevelopmental psychopathology. PLoS. One. (2011)

[5] Z. Wen, H.N. Nguyen, Z. Guo, M.A. Lalli, X. Wang, Y. Su, N.S. Kim, K.J. Yoon, J. Shin, C. Zhang, G. Makri, D. Nauen, H. Yu, E. Guzman, C.H. Chiang, N. Yoritomo, K. Kaibuchi, J. Zou, K.M. Christian, L. Cheng, C.A. Ross, R.L. Margolis, G. Chen, K.S. Kosik, H. Song, G.L. Ming, Synaptic dysregulation in a human iPS cell model of mental disorders, Nature. 515 (2014) 414-8.

ACCEPTED MANUSCRIPT

[6] C.M. O'Tuathaigh, J.L. Waddington, Closing the translational gap between mutant mouse models and the clinical reality of psychotic illness, Neurosci. Biobehav. Rev. 58 (2015) 19-35.

[7] P.A. Leighton, K.J. Mitchell, L.V. Goodrich, X. Lu, K. Pinson, P. Scherz, W.C. Skarnes, M. Tessier-Lavigne, Defining brain wiring patterns and mechanisms through gene trapping in mice, Nature 410 (2001) 174-9.

[8] G. Kerjan, J. Dolan, C. Haumaitre, S. Schneider-Maunoury, H. Fujisawa, K.J. Mitchell, A. Chedotal, The transmembrane semaphorin Sema6A controls cerebellar granule cell migration, Nat. Neurosci. 8 (2005) 1516-24.

[9] F. Suto, M. Tsuboi, H. Kamiya, H. Mizuno, Y. Kiyama, S. Komai, M. Shimizu, M. Sanbo, T. Yagi, Y. Hiromi, A. Chedotal, K.J. Mitchell, T. Manabe, H. Fujisawa, Interactions between Plexin-A2, Plexin-A4, and Semaphorin 6A control lamina-restricted projection of hippocampal mossy fibers. Neuron. 53 (2007) 535-47.

[10] G.E. Little, G. Lopez-Bendito, A.E. Runker, N. Garcia, M.C. Pinon, A. Chedotal, Z. Molnar, K.J. Mitchell, Specificity and plasticity of thalamocortical connections in Sema6A mutant mice, PLoS. Biol. 7 (2009) e98.

[11] A.E. Rünker, G.E. Little, F. Suto, H. Fujisawa, K.J. Mitchell, Semaphorin-6A controls guidance of corticospinal tract axons at multiple choice points, Neural. Dev. 3 (2008) 34.

ACCEPTED MANUSCRIPT

[12] F. Perez-Branguli, Y. Zagar, D.K. Shanley, I.A. Graef, A. Chédotal, K.J. Mitchell, Reverse signaling by semaphorin-6A regulates cellular aggregation and neuronal morphology, PLoS. One. 11 (2016) e0158686.

[13] J. Gilabert-Juan, A.R. Sáez, G. Lopez-Campos, N. Sebastiá-Ortega, R. González-Martínez, J. Costa, J.M. Haro, L.F. Callado, J.J. Meana, J. Nacher, J. Sanjuán, M.D. Moltó, Semaphorin and plexin gene expression is altered in the prefrontal cortex of schizophrenia patients with and without auditory hallucinations, Psychiatry. Res. 229 (2015) 850-7.

[14] J.C. Crabbe, R.G. Morris, Festina lente: late-night thoughts on high-throughput screening of mouse behaviour. Nat. Neurosci. 7 (2001) 175-79.

[15] J.L. Waddington, C. O'Tuathaigh, G. O'Sullivan, K. Tomiyama, N. Koshikawa, D.T. Croke, Phenotypic studies on dopamine receptor subtype and associated signal transduction mutants: insights and challenges from 10 years at the psychopharmacology-molecular biology interface. Psychopharmacology 181 (2005) 611-38.

[16] C.M. O'Tuathaigh, G.J. O'Sullivan, A. Kinsella, R.P. Harvey, O. Tighe, D.T. Croke, J.L. Waddington, Sexually dimorphic changes in the exploratory and habituation profiles of heterozygous neuregulin-1 knockout mice. NeuroReport 17 (2006) 79-83.

ACCEPTED MANUSCRIPT

[17] J. Walsh, L. Desbonnet, N. Clarke, J.L. Waddington, C.M.P. O'Tuathaigh. Disruption of exploratory and habituation behaviour in mice with mutation of DISC1: an ethologically-based analysis. J. Neurosci. Res. (2012) .

[18] D. Babovic, C.M. O'Tuathaigh, G.J. O'Sullivan, J.J. Clifford, O. Tighe, D.T. Croke, M.K. Karayiorgou, J.A. Gogos, J.L. Waddington, Exploratory and habituation phenotype of heterozygous and homozygous COMT knockout mice, Behav. Brain. Res. 183 (2007) 236-9.

[19] R.E. Nally, A. Kinsella, O. Tighe, D.T. Croke, A.A. Fienberg, P. Greengard, J.L. Waddington, Ethologically based resolution of D2-like dopamine receptor agonist- versus antagonist-induced behavioral topography in dopamine- and adenosine 3',5'-monophosphate-regulated phosphoprotein of 32 kDa "knockout" mutants congenic on the C57BL/6 genetic background. J. Pharmacol. Exp. Ther. 310 (2004) 1281-7.

[20] S.O. Shim, W.B. Cafferty, E.C. Schmidt, B.G. Kim, H. Fujisawa, S.M. Strittmatter, PlexinA2 limits recovery from corticospinal axotomy by mediating oligodendrocyte-derived Sema6A growth inhibition, Mol. Cell. Neurosci. 50 (2012) 193-200.

[21] J.A. Gogos, M. Morgan, V. Luine, M. Santha, S. Ogawa, D. Pfaff, M. Karayiorgou, Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behaviour. Proc. Natl. Acad. Sci. USA. 95 (1998) 9991-6.

ACCEPTED MANUSCRIPT

[22] A. Messina, P. Giacobini, Semaphorin signaling in the development and function of gonadotrophin hormone-releasing hormone system. Front. Endocinol. 4 (2013) 133.

[23] K.E. Stephan, K.J. Friston, C.D. Frith, Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. Schizophr. Bull. 35 (2009) 509-27.

[24] P.F. Whitty, O. Owoeye, J.L. Waddington, Neurological signs and involuntary movements in schizophrenia: intrinsic to and informative on systems pathobiology, Schizophr. Bull. 35 (2009) 415-24.

[25] I. Ellison-Wright, E. Bullmore, Anatomy of bipolar disorder and schizophrenia: a meta-analysis, Schizophr. Res. 117 (2010) 1-12.

[26] J.A. Pratt, B.J. Morris, The thalamic reticular nucleus: a functional hub for thalamocortical network dysfunction in schizophrenia and a target for drug discovery, J. Psychopharmacol. 29 (2015) 127-37.

[27] G. Douaud, S. Smith, M. Jenkinson, T. Behrens, H. Johansen-Berg, J. Vickers, S. James, N. Voets, K. Watkins, P.M. Matthews, A. James, Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia, Brain. 130 (2007) 2375-86.

ACCEPTED MANUSCRIPT

[28] N.C. Andreasen, R. Pierson, The role of the cerebellum in schizophrenia, Biol. Psychiatry. 64 (2008) 81-88.

[29] H. Picard, I. Amado, S. Mouchet-Mages, J.P. Olie, M.O. Krebs, The role of the cerebellum in schizophrenia: an update of clinical, cognitive, and functional evidences, Schizophr. Bull. 34 (2008) 155-72.

[30] M. Geyer, B. Moghaddam, Animal models relevant to schizophrenia disorder. In: K.L. Davis, C. Charney, J.T. Coyle, C. Nemeroff (eds), Psychopharmacology: The Fifth Generation of Progress. Lippincott Williams and Wilkins, 2002: Philadelphia, pp 689-701.

[31] M. Van den Buuse, Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophr. Bull. 36 (2010) 246-70.

[32] T.V. Lipina, J.C. Roder, Disrupted-In-Schizophrenia-1 (DISC1) interactome and mental disorders: impact of mouse models, Neurosci. Biobehav. Rev. 45 (2014) 271-94.

ACCEPTED MANUSCRIPT

Fig. 1. Behavioural counts for locomotion, sniffing, total rearing, rearing to wall, rearing free, rearing seated, total grooming, sifting and chewing in male (M) and female (F) Sema6A WT, HET, and KO mice. Data are mean counts ± SEM over a 60 min period of initial exploration. ∗ $P < 0.05$ vs. WT & HET.

Fig. 2. Behavioural counts for locomotion, sniffing, total rearing, rearing seated, rearing to wall and sifting in Sema6A WT, HET and KO mice of both sexes. Data are mean counts ± SEM over a 370 min period of habituation. For statistical analysis, see text.

Fig. 3. Latency to fall in the accelerating rotarod task in (A) male and (B) female (F) Sema6A WT, HET and KO mice. Data are mean counts ± SEM over six successive days of training. For statistical analysis, see text.

ACCEPTED MANUSCRIPT

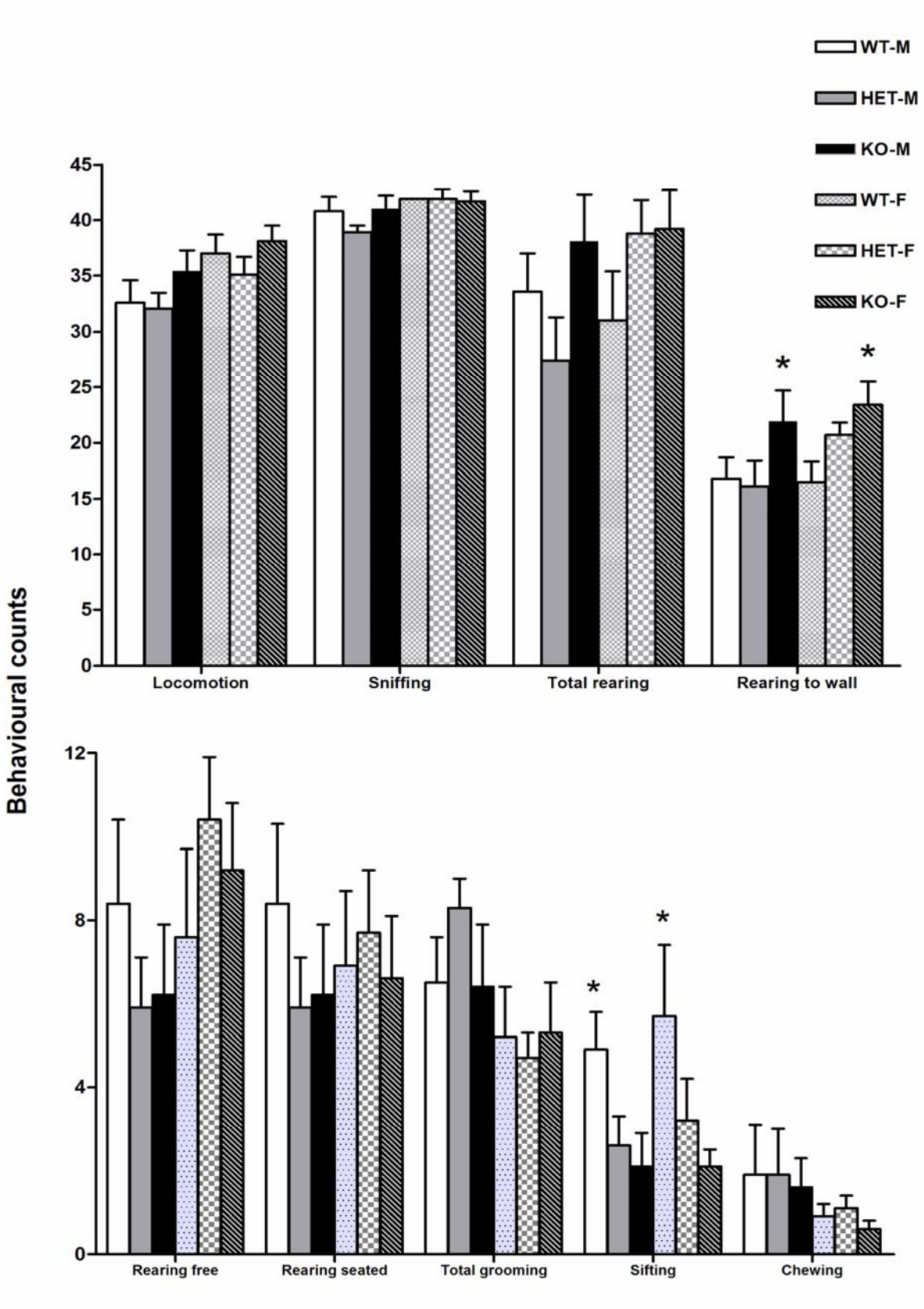


Fig 1

ACCEPTED MANUSCRIPT

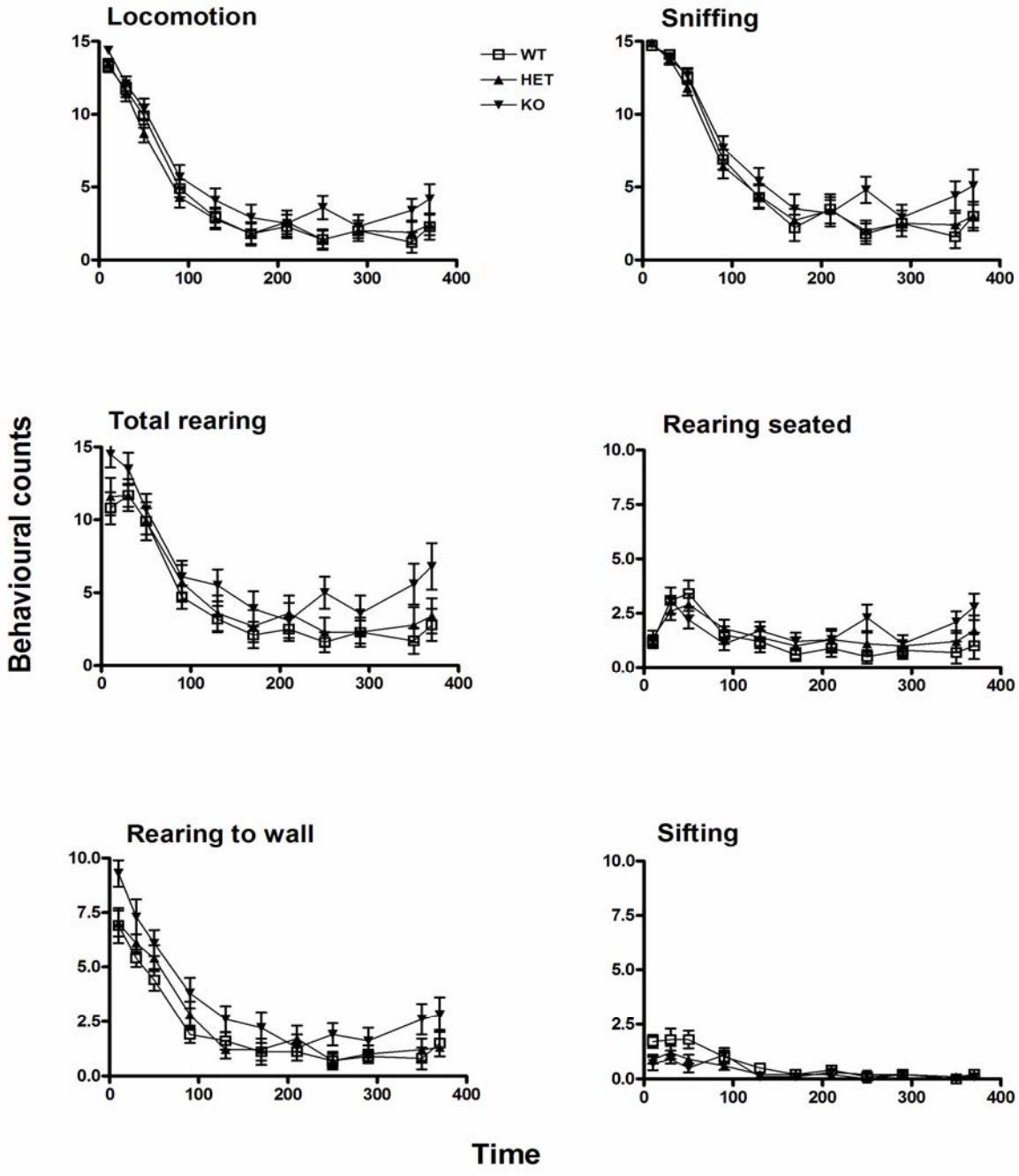


Fig 2

ACCEPTED MANUSCRIPT

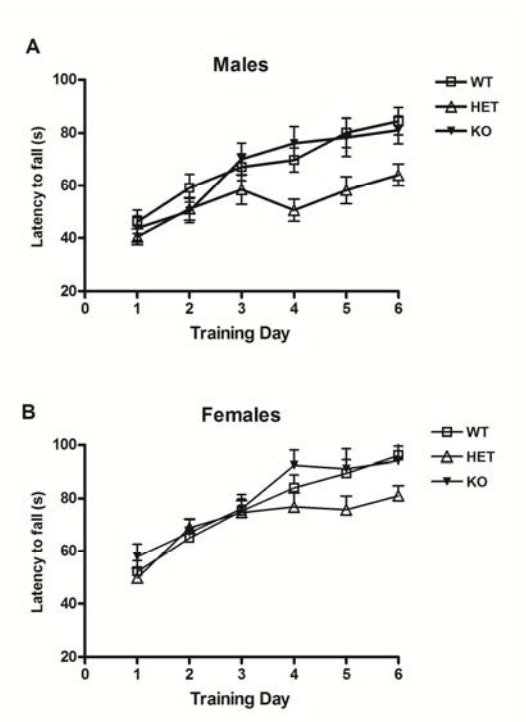


Fig 3