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Research article

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

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HIGHLIGHTS

- Genetic disruption of 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.

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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.

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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

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catechol-*O*-methyl-transferase [COMT; 18]. This approach involves 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 °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.

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) 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. 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.

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, $F(10,520) = 6.18$, $P < 0.001$],

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