Beta N-acetylglucosaminyltransferase V (Mgat5) deficiency reduces the depression-like phenotype in mice

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The central nervous system (CNS) is rich in glycoconjugates, located on cell surface and in extracellular matrix. The products of Golgi UDP-GlcNAc:N-acetylglucosaminyltransferases (encoded by Mgat1, Mgat2, Mgat4 and Mgat5) act sequentially to generate the GlcNAc-branched complex-type N-glycans on glycoprotein receptors. While elimination of all the branched N-glycans in Mgat1−/− mouse embryos is lethal at neural tube fold stage, decreased branching is associated with late developmental defects similar to type 2 of congenital disorders of glycosylation, with developmental and psychomotor abnormalities. To study the role of complex-type N-glycans in brain function, we tested Mgat5−/− mice in a battery of neurological and behavioral tests. Despite the absence of tri- and tetra-antennary products, Mgat5−/− mice were not different from their wild-type littermates in physical and neurological assessments, anxiety level, startle reactivity and sensorimotor gating. However, they displayed a robust decrease in locomotor activity, interpreted as a change in depression-like behavior. This effect was accentuated after chronic mild stress. Comparable increase in plasma corticosterone of Mgat5−/− and Mgat5−/− mice in response to acute stress shows an intact function of the hypothalamus–pituitary–adrenal axis. A change in social interactions was also observed. Our results indicate that Mgat5 modification of complex-type N-glycans on CNS glycoproteins is involved in the regulation of depression-like behavior.

Keywords: Animal models, complex-type N-glycans, depression-like behavior, Mgat5 knockout mice, stress

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Brain development and function are dependent on cell–substratum and cell–cell interactions mediated by surface receptors and transporters, which are generally glycoproteins (Kleine & Schachner 2004). The glycosylation process starts in the endoplasmic reticulum and continues in the Golgi apparatus, where N-acetylglucosaminyltransferases (encoded by Mgat1, Mgat2, Mgat4 and Mgat5) sequentially add GlcNAc branches and increase the affinities for N-acetylactosamine-binding galectins (Hirabayashi et al. 2002; Partridge et al. 2004).

The complex type N-glycans are required for the development of the embryo, and their complete absence in Mgat1-deficient mouse is lethal with defects in neural tube formation (loffee 1994). Moreover, neuron-specific Mgat1 null mutation results in failure to thrive in mice and death by 8 weeks postnatal (Ye & Marth 2004).

The decreased level of bi-, tri- and tetra-antennary N-glycans in Mgat2−/− mice models the congenital disorders of glycosylation-IIa (Tan et al. 1996; Wang et al. 2001) in humans, which manifest as severe dysmorphic and multi-systemic defects, psychomotor abnormalities and mental retardation (Freeze 2002; Tan et al. 1996).

Mgat5 is widely expressed in the post-E9.5 mouse embryo and highly expressed in the adult CNS. Although Mgat5-deficient mice are healthy at birth, they lack tri–tetra-antennary N-glycans and display adult phenotypes including increased susceptibility to autoimmune disease and reduced cancer progression (Demetriou et al. 2001; Dennis et al. 2002; Granovsky et al. 2000).

The activity-dependent generation of optical dominance columns is tightly regulated by the extracellular matrix (Berardi et al. 2003, 2004). The removal of chondroitin sulfate proteoglycans with chondroitinase shifts the ocular dominance columns (Pizzorusso et al. 2002) toward the nondeprived eye even in adult rats (Pizzorusso et al. 2006). Because the pattern of glycosylation is altered in Mgat5 mutant mice, we decided to scan these mice with an array of behavioral tasks that depend on activity-dependent plasticity in the rest of the brain.

Here, we characterized Mgat5−/− mice in neurological and behavioral assays including tests of emotionality, the depression-like state, sensorimotor gating and cognitive performance. We find that Mgat5-deficient mice were normal in these tasks but had altered depression-like behavior.

Materials and methods

Animals

Mgat5−/− mice were previously generated by Granovsky et al. (2000) and had been backcrossed for nine generations to C57BL/6 (B6)
behind. Mgat5<sup>-/-</sup> mice and their Mgat5<sup>WT/+</sup> littermate controls were obtained from heterozygous mating. The pups of mixed genotypes were weaned at 4 weeks of age and housed by sex in groups of two to five in cages with wood chip bedding. Food (Purina mouse chow) and water were provided ad libitum unless otherwise mentioned (see protocol for chronic mild stress). The vivarium temperature was maintained at 21 ± 1°C, with humidity at 50–60%. The 12:12 h light–dark cycle was maintained with artificial light (lights on from 0700 to 1900 h).

Behavioral testing

All the experiments were conducted during the light phase from 0900 to 17:00 h. Cohorts of Mgat5<sup>-/-</sup> mice and wild-type littermate controls were used in the experiments. Unless otherwise mentioned, male and female subjects were not different in the behavior of interest, and the relevant data were pooled for more subject numbers. The subjective tests (i.e. open field, OF, elevated plus maze, EP, social interaction, forced swim test (FST) and tail suspension test (TST)) were videotaped. Random trials were rescored by a second observer, which was blind to the genotype of the mice, to ensure that the scorings were not biased. All the experiments were conducted in accordance with the requirements of the Province of Ontario ‘Animals for Research Act, 1971’ and the Canadian Council on Animal Care.

Experiment 1 (behavioral phenotyping)

The experiments started when the mice were 7–8 weeks of age in the following order: preliminary physical examination, EP, OF, social interaction, Morris water maze, startle reactivity and prepulse inhibition (PPI) of startle, rotarod, hidden cookie test, FST, TST and fasted FST. The mice were given minimum 7-day interval between the tests. Prior to all experiments, mice were left undisturbed in the room for 30 min to allow acclimation.

Physical and neurological assessment

Physical examination. A physical examination was performed on adult mice, which included measuring weight, assessing presence or absence of whiskers, fur condition, eye blink, ear twitch, whisker twitch and righting reflex (Miyakawa et al. 2001). The weight was remeasured when the mice were 14–18 weeks old.

Hidden cookie test. The ability of a fasted mouse to locate a buried piece of peanut cookie was used to measure the olfactory sensitivity, as previously explained by Wrenn et al. (2003). In order to familiarize the mice with the food, they were fed with peanut cookie after overnight fasting. Twenty-four hours later, the subjects were food deprived overnight. The test was conducted in a clean mouse cage, where a small cube of peanut cookie (5 mm on each side) was randomly buried underneath 2 cm of clean bedding. The latency to find and eat the cookie was recorded by the observer.

Accelerating rotarod. For this experiment, an Economex Rotarod apparatus was used (Columbus Instruments, Columbus, OH, USA). The original 3-cm ribbed plastic rotating axle was divided into four adjustable flanges, which enabled testing a maximum of four mice simultaneously. The rod was suspended at a height of 30 cm above the plastic surface. Mice were placed on top of the rod, facing away from the experiment. In this orientation, forward locomotion opposite to rotation of the rod was necessary to avoid falling. During the stationary mode, each mouse was first observed for 60 seconds without any rotation. The axe was then adjusted for a constant motor speed of 5 r.p.m., and each mouse was observed for a total of 90 seconds (fixed speed mode). Next, beginning at 5 r.p.m., the rotation gradually increased in increments of 0.1 r.p.m. every second and the latency to fall off the axe was recorded in seconds for each mouse for the maximum period of 300 seconds (accelerating speed model). The mean latency was then calculated by averaging the latency for three consecutive trials (Abramow-Newerly et al. 2006). The stationary and fixed speed mode sessions were training periods, allowing the animals to become accustomed to the apparatus. Impaired performance in these sessions served as early indicators of abnormality.

Tests of emotionality and general activity

Elevated plus maze. The test apparatus was made as described in Augustinovich et al. (2000). The maze consisted of two open arms (25 × 5 cm), two enclosed arms (25 × 5 × 30 cm) and a central platform (5 × 5) arranged so that similar arms were opposite each other and at right angles to dissimilar arms. The arms and the floor were constructed from the opaque Plexiglas material and were elevated 50 cm above the floor. Experiments were conducted in a dimly lit room, the light intensity on the central platform was 210 lux. Subjects for this test were about 8 weeks old and had only been previously tested in a brief physical exam (above). The apparatus was cleaned with 70% ethanol between subjects. Each mouse was placed in the center of the maze (5 × 5) facing the closed arm. Data collection was performed by Observer version 5.0 software (Noldus Information Technology, Wageningen, the Netherlands). During a 6-min observation period, the number of entries (defined as four paws into an arm) and the amount of time spent in open arm, closed arm and the central platform were scored by the observer. The total number of entries for each subject was collected. These data are presented as percentage spent in the closed and open arm (i.e. time spent in closed or open arm/total duration of experiment × 100).

Open field. Mice were individually placed in the middle of the low-lit (light intensity was 120 lux) automated activity cage (41 × 41 × 33 cm<sup>3</sup>) (model 7420/7430; Ugo Basile, Comerio VA, Italy), and horizontal and vertical activity was measured for 30 min. The arena was cleaned with 70% ethanol solution between subjects.

Social interaction test in neutral cage. Male mice were used to estimate social behavior. Each mouse was placed in an illuminated (280 lux) and unfamiliar neutral cage (30 × 17 × 12 cm), as previously described (File 2001), and the social interaction was scored when the mouse encountered a weight- and age-matched unfamiliar adult wild-type B6 male (standard opponent). Standard opponents were used only once, and neutral cages were changed for each tested pair. The observation period started with the first interaction of the mice and lasted for 5 min. During this period, the following behaviors of the experimental animal were recorded: social investigation including stretched attend, sniffing of partner following genital grooming, flight (as an active avoidance of partner), freezing (passive sitting with slight movements of head), aggressive (threat, attack, bite, chase and aggressive grooming) and nonsocial behavior such as self-grooming, cage exploration, digging and rearing.

Acoustic startle response and PPI

Prepulse inhibition was conducted in four isolation chambers (Startle Reflex System, ENV-022s, MED Associates Inc., St Albans, VT, USA), which were foam lined in order to dampen the sound and were equipped with a ventilation fan and red light. Located in each chamber were an acoustic stimulator (ANL-92S), a transducer amplifier (PHM-255A and PHM-250B) and an animal holder that was mounted on the top of the platform. The grid floor holder was large enough to allow the animal adequate movement and ventilation. The platform detected and transduced the motion of the animal. All events were recorded and controlled by MED Associates software (Startle Reflex package). The holders were cleaned with 70% ethanol between trials.

Acoustic startle reactivity. During the procedure, the animal was confined to the holder with a background noise of 65 dBA. To test startle reactivity, three blocks, each composed of 10 pseudorandomly organized tones (70, 75, 80, 85, 90, 95, 100, 105, 110, 115 and 120 dB), were presented with 50 milliseconds duration and interstimulus intervals of 20–30 seconds. The peak startle activity in each trial
was dissolved and further sonicated in 0.9% saline solution on the
which decreases the immobility time in the TST and the FST (David
are sensitive to desipramine hydrochloride, a tricyclic antidepressant,
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et al.

Tests of depression-like behavior
Forced swim test. The protocol was performed as described by
Cryan et al. (2002). The mice were released individually into a trans-
parent plastic cylinder (25 cm height, 18 cm diameter), which con-
tained water at 25°C to the depth of 18 cm. The experiment lasted
6 min, and an observer scored the following parameters in the last
4 min of the trial using Observer 5.0 (Noldus Information Technology):
(1) latency, frequency and duration of each event: climbing (upward-
directed movements of the forepaws along the side of the swimming
chamber), (2) active swimming (including crossing the quadrants of
the container) and (3) floating (no limb movement and making only
minimal movements to keep the head above the water). Each mouse
was allowed to dry after the test, and the water was changed
between subjects.

Tail suspension test. This procedure was followed as previously
described by Steru et al. (1985). Scotch tape pasted to the tip of the
tail (~1 cm) was used to securely fasten the mice to a flat wooden
surface located 50 cm above the ground. Active hanging and immo-
ibility, defined by presence or absence of limb movement, were
recorded by the observer over a 5-min period.

Fasted FST. Mgat5−/− mice displayed a slight weight deficit as
well as 10% lower basal blood glucose level than their littermate
controls, under both fed and fasting conditions (Lau et al. 2007). As
caloric restriction is associated with hyperactivity (Overton & Williams
2004; Williams et al. 2002), we wanted to investigate if acute
exacerbation of hypoglycemia had an effect on the FST. The FST
was repeated 24 h after the food pellets were removed from the
home cage to check if food deprivation aggravated the active
swimming in the FST.

After the first set of experiments, more behavioral tests were
designed to explore the characteristics of the decreased immobility.
Separate groups of mice were tested in two sets of experiments.

Experiment 2 (effect of antidepressant treatment on
immobility duration in TST)
The C57BL/6 background used in our experiments is limited in
sensitivity to serotonin-specific reuptake inhibitors (SSRI) (David
et al. 2003; Lucki et al. 2001). However, the C57BL/6 strain mice
are sensitive to desipramine hydrochloride, a tricyclic antidepressant,
which decreases the immobility time in the TST and the FST (David
et al. 2003; Ripoll et al. 2003).

Desipramine hydrochloride (20 mg/kg; Sigma, St Louis, MO, USA)
was dissolved and further sonicated in 0.9% saline solution on the
experiment days and administered intraperitoneal (i.p.), 30 min before
the TST, in a volume of 10 ml/kg. The dose of desipramine hydro-
chloride was chosen based on literature (Holmes et al. 2002) and our
pilot study on C57BL/6J mice (data not shown). The TST was chosen
over the FST, as the former generated clear-cut results in the dose
response experiments.

Experiment 3 (effect of chronic mild stress on
immobility duration in FST and TST)
In the previous experiment, we found low immobility in FST and TST
in Mgat5−/− mice, which suggested an active strategy for more
coping under highly stressful situations. We hypothesized that
Mgat5−/− mice with ‘more active’ strategy would be exhausted
faster in prolonged stress, resulting in a depressive-like behavior.
Three days after the last stressors, the FST and the TST were
performed on the mice on two consecutive days. Performance of
each mouse before (trial 1) and after (trial 2) stress was compared for
floating in FST as well as immobile hanging in TST. The intertrial
difference in immobility (Δimmobility) was calculated as duration in the trial
2 – duration in the trial 1.

Chronic mild stress (CMS) protocol
All subjects were previously tested in experiment 1. The protocol for
chronic mild stress was adopted from Duchet et al. (2003) and was
modified to adjust to our animal facility. The protocol consisted of
randomized daily stressors of different types (two stressors per day)
for 24 days followed by retesting in the FST and TST to measure level of
depression (Bielajew et al. 2003; Tannenbaum et al. 2002). The
stressors included physical restraint, crowding the cages with the
bedding, 30°C tilting the cage, shocking, 15-h food and water
restriction and 5-min forced swimming.

Physical restraint. Each mouse was individually placed in a plastic
decapitone (Braintree scientific inc., MA, USA) for 1 h
in a horizontal position. The animals were capable of breathing
through the breathing hole at the smallest end and were immobilized
without being squeezed. The larger end was tightly closed with
a paper clip. All the subjects were checked to prevent suffocation.

Cage crowding with wet bedding. Subjects of the same
gender from different cages are introduced to a soaked home cage
(about 200 ml of water for 100 gm of bedding) or a more aggressive
mouse strain (FVB) (Pugh et al. 2004). Ten animals were used per
cage. The cages were closely observed during the 10-min experiment
to stop aggression among the mice.

Shocking. The mice were placed in the fear conditioning chambers
(20.5 × 21.1 × 21.0 cm) (MED Associates). Each trial consisted of
a 2-min acclimation period, two shocks (1 second, 0.35 mA), with a 2-
min interval preceded by a 20-second auditory stimulus. Two trials of
shocks (total of four shocks) were administered on each stress
session.

Hormonal measurements
Stress stimulates the hypothalamus–pituitary–adrenal (HPA) axis,
resulting in elevated serum corticosterone. To determine whether
the HPA response to stress is altered in Mgat5−/− mice, basal and
stressed level of corticosterone was measured as previously
described (Karanth et al. 1997). Hormonal measurements were
performed between 0900 and 1100 h. Both groups were single
housed for 1 week prior to the assay. The stressed controls were
physically restrained for 30 min in plastic film decapitones (Braintree
scientific inc.), while the non-stressed mice were undisturbed. Mice
were individually anesthetized in a glass jar saturated with Halothane
(Halocarbon laboratories. River Edge, NJ, USA) followed by cardiac
puncture. In order to get reliable basal values, the nonstressed mice
were anesthetized in less than 15% halothane after the first cage
manipulation and the sampling process was accomplished within 2–3 min. Blood was collected in ice-chilled ethylenediaminetetraacetic acid tubes, and the plasma was separated by centrifugation (3900 g for 5 min) and stored at -20 °C. Plasma corticosterone level was quantified by enzyme-linked immunosorbent assay (Neogen, Lansing, MI, USA).

Statistical analyses

Statistical analyses of the behavioral tests were performed by using analysis of variance (ANOVA), with gender and genotype as between-subject variables. Tukey’s honest significant difference (HSD) post hoc analysis was used when ANOVAs yielded statistically significant main or interaction effects. Whenever there was no effect of gender, male and female data were presented together. The t-test was used to compare the performance of male subjects in the social interaction test. In startle reactivity, the data were analyzed with two-way ANOVA, with gender and genotype as between-subject variables and intensities as repeated measures within subject variable. Probabilities of <0.05 were considered significant.

To examine the effect of weight on immobility duration in startle reactivity, PPI, FST and TST, an additional analysis of covariance (ANCOVA) was conducted among the genotypes, with weight as a covariate. Using the Kolmogorov–Smirnov test, the normality of weight was analyzed.

All statistics were calculated by Statistica for Windows 5.5 (StatSoft, Tulsa, OK, USA). All the values reported in the texts and figures are expressed as means ± SEM.

Table 1: Summary of behavioral results in the Mgat5−/−. Except for the development of weight deficit and the robust decreased immobility in the depression tests, Mgat5−/− mice were normal in most phenotypic parameters. Unless otherwise mentioned, male and female subjects were added

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Mgat5+/+</th>
<th>Mgat5−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance and neurological exams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, g (14–18 weeks)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Females</td>
<td>20.90 ± 0.48</td>
<td>19.11 ± 1.23</td>
</tr>
<tr>
<td>Males</td>
<td>25.19 ± 0.71</td>
<td>23.67 ± 0.36</td>
</tr>
<tr>
<td>Olfaction, seconds (latency to find hidden cookie)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Females</td>
<td>35.85 ± 4.84</td>
<td>33.5 ± 6.6</td>
</tr>
<tr>
<td>Males</td>
<td>125.11 ± 8.47</td>
<td>116 ± 5.14</td>
</tr>
<tr>
<td>Motor co-ordination, rotarod, seconds (latency to fall)</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Females</td>
<td>28.33 ± 2.41</td>
<td>39.62 ± 6.64</td>
</tr>
<tr>
<td>Males</td>
<td>38.14 ± 5.6</td>
<td>26.75 ± 4.21</td>
</tr>
<tr>
<td>Close arm entries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>11.16 ± 0.92</td>
<td>15 ± 2.07</td>
</tr>
<tr>
<td>Males</td>
<td>13.57 ± 2.02</td>
<td>11.25 ± 1.89</td>
</tr>
<tr>
<td>% Time in open arms</td>
<td></td>
<td></td>
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<tr>
<td>Females</td>
<td>3.53 ± 1.07</td>
<td>3.81 ± 1.03</td>
</tr>
<tr>
<td>Males</td>
<td>4.5 ± 1.24</td>
<td>2.07 ± 0.84</td>
</tr>
<tr>
<td>% Time in close arms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>89.89 ± 1.15</td>
<td>88.08 ± 2.13</td>
</tr>
<tr>
<td>Males</td>
<td>84.14 ± 3.98</td>
<td>91.43 ± 1.59</td>
</tr>
<tr>
<td>Average % PPI</td>
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<tr>
<td>Females</td>
<td>42.32 ± 7.36</td>
<td>-44.54 ± 7</td>
</tr>
<tr>
<td>Males</td>
<td>-54.02 ± 5.14</td>
<td>-44.54 ± 7</td>
</tr>
<tr>
<td>Average startle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>613.14 ± 79.25</td>
<td>382.74 ± 55.30</td>
</tr>
<tr>
<td>Males</td>
<td>960.86 ± 102.76</td>
<td>741.98 ± 104.49</td>
</tr>
</tbody>
</table>

**Mgat-5 mutants show altered depression-like behavior**

**Results**

**Physical and neurological assessments**

Young adult Mgat5−/− mice (7–8 weeks) were physically indistinguishable from their wild-type littermates. Distribution of weight was normal in all groups. When reassessed at 14–18 weeks of age, mutant mice had developed a weight deficit (main effect of genotype: \( F_{1,41} = 5.11; P < 0.05 \) and main effect of gender: \( F_{1,41} = 36.59; P < 0.0001 \)). Fur condition, reflexes and olfaction, tested in the hidden cookie test, were also not different between genotypes (main effect of genotype: \( F_{1,26} = 1.09; P > 0.1 \)). Latency to fall from the accelerating rotating rod, which is an indicator of motor co-ordination, was also not different between Mgat5−/− and Mgat5+/+ mice (main effect of genotype: \( F_{1,36} = 0.53; P > 0.1 \) (Table 1).

**Depression-like behavior**

**FST and TST**

In the FST, Mgat5−/− mice (\( n = 34 \)) showed more active swimming than Mgat5+/+ mice (\( n = 34 \)) (main effect of genotype: \( F_{1,66} = 34.10, P < 0.0001 \)) and spent less time floating (Fig. 1a; main effect of genotype: \( F_{1,66} = 88.74, P < 0.0001 \)).
Mgat5+/+ Mgat5–/– Mgat5+/+

P < 0.0001). In the same manner, Mgat5–/– mice of both genders (n = 18) were significantly more active in the TST test compared with their wild-type littermates (n = 16) (Fig. 1b; main effect of genotype: F1,30 = 55.83, P < 0.0001). Additional analysis of covariance analysis of the results with weight as a covariate showed that the main effect of genotype remained highly significant in TST (F1,42 = 67.95, P < 0.0001) and FST (F1,15 = 51.16, P < 0.0001), and weight differences did not affect the immobility duration in either test (P > 0.1 and P > 0.5, respectively).

To check if prior testing of mice affected the depression tests, a group of naive subjects were tested in FST (Mgat5+/+, n = 8 and Mgat5–/+; n = 13) and TST (Mgat5+/+, n = 5 and Mgat5+/+; n = 7). Results showed that the immobility remained significantly low in naive Mgat5–/– mice in both tests (F1,12 = 24.93, P < 0.0001 for FST and F1,8 = 63.24, P < 0.0001 for TST).

Effect of fasting on the FST
To determine whether fasting and the resulting hypoglycemia in Mgat5–/– mice could exacerbate the decreased immobility, the FST was repeated after 24 h food deprivation and compared with the nonfasted controls (Mgat5–/– fasted, n = 9; Mgat5+/– nonfasted, n = 5; Mgat5+/+ fasted, n = 10; Mgat5+/+ nonfasted, n = 5). No effect of food deprivation on floating duration was observed (P > 0.1), and the genotype effect remained significant (F1,25 = 34.49, P < 0.0001).

Effect of antidepressant treatment on TST
Desipramine injection decreased floating duration in Mgat5+/+ mice (desipramine, n = 13 and saline, n = 11) (Fig. 2; main effect of drug treatment: F1,39 = 17.966, P < 0.0001), while it had no significant effect on Mgat5–/– mice (desipramine, n = 12 and saline, n = 11), which were already highly mobile. Mgat5–/– mice had lower immobility compared with Mgat5+/+ mice in both drug- and saline-treated groups (main effect of genotype: F1,39 = 37.852, P < 0.0001).

CMS
The FST and TST were repeated after the CMS to evaluate the impact of chronic stress on the depression-like behavior. The difference in immobility duration between trials (Δimmob) was significantly different between mutant (n = 10) and their wild-type littermates (n = 7) in both FST (Fig. 3b; main effect of genotype on Δimmob floating duration: F1,15 = 4.65, P < 0.05) and TST (Fig. 3d; main effect of genotype on Δimmob inactive hanging duration: F1,15 = 4.24, P < 0.05). Mgat5+/+ mice numerically increased their immobility duration after CMS in the FST and TST. Mgat5–/–, on the other hand, became more active in both tests.

Other behavioral tests
Tests of emotionality and general activity
Horizontal and vertical activity recorded by the automated OF apparatus during the 30-min encounter with the dimly lit arena was not different between the genotypes (Mgat5+/– female, n = 6; Mgat5–/– male, n = 6; Mgat5+/+ female, n = 5 and Mgat5+/+ male, n = 5) (Fig. 4a,b; main effect of genotype on horizontal and vertical activity: F1,18 = 0.24, P > 0.1 and F1,18 = 0.18, P > 0.1, respectively). However, females were more active in horizontal and vertical activity (Fig. 4; main effect of gender on horizontal and vertical activity: F1,18 = 13.50, P < 0.0001).

Figure 1: Forced swim test and TST. As there was no significant effect of gender, male and female data were pooled. Mgat5–/– mice spent (a) significantly less time floating (post hoc P < 0.0001) when tested in the FST and (b) were more active in the TST (post hoc P < 0.0001). Solid bars denote wild-type values, and striped bars represent knockout values. Forced swim test: Mgat5–/–, n = 34; Mgat5+/+, n = 34; TST: Mgat5–/–, n = 18; Mgat5+/+, n = 16 (**P < 0.001).

Figure 2: Tail suspension test, following desipramine treatment. Twenty milligram per kilogram desipramine resulted in significant decrease in immobility in Mgat5+/+ mice (post hoc P < 0.001) but not the Mgat5–/– mice. Drug- and saline-treated Mgat5–/– had significant lower immobility compared with their control wild types (post hoc P < 0.01 and P < 0.0001, respectively). As no significant effect of gender was found, males and females were pooled. Solid bars denote wild-type values, and striped bars represent knockout values. Mgat5–/– desipramine, n = 12; Mgat5–/– saline, n = 11; Mgat5+/+ desipramine, n = 13 and Mgat5+/+ saline, n = 11. (**P < 0.01 and ***P < 0.001).
Mgat-5 mutants show altered depression-like behavior

Figure 3: Forced swim test and TST following chronic stress. Mgat5+/+ mice increased their floating duration (a,b) and inactive hanging (c,d) after CMS, while Mgat5−/− decreased the immobility in both tests (post hoc P < 0.05). As no significant effect of gender was found, males and females were pooled. Squares and solid bars denote Mgat5−/− mice, and diamonds and open bars denote Mgat5+/+ mice. Mgat5−/−, n = 10; Mgat5+/+, n = 7 (*P < 0.05).

activity: $F_{1,18} = 30.53, P < 0.01$ and $F_{1,18} = 6.2, P < 0.05$, respectively).

In the EP, there was no statistical difference between the two genotypes (Mgat5−/− female, n = 8; Mgat5−/− male, n = 8; Mgat5+/+ female, n = 12 and Mgat5+/+ male, n = 14) in the number of entries to all arms and to the closed arms (main effect of genotype on all arms and closed arms: $F_{1,36} = 0.0001, P > 0.1$ and $F_{1,36} = 0.16, P > 0.1$, respectively), which are the main measurements of activity in this test (Rodgers & Johnson 1995). The percentage of time in the open and closed arms, variables that correlated with anxiety state (Rodgers & Johnson 1995), were not significantly different between the genotypes (main effect of genotype on per cent open and closed arm: $F_{1,36} = 0.69, P > 0.1$ and $F_{1,36} = 0.81, P > 0.1$, respectively).

In the test of social interaction, Mgat5−/− males (n = 8) spent less time sniffing the opponent (Fig. 5a; $P < 0.05$) compared with the wild-type littermates (n = 6). However, they showed an increased cage exploration (Fig. 5b; $P < 0.01$) and a borderline increase in digging in the cage bedding (Fig. 5c; $P = 0.06$). Other variables were not significantly different between the two groups.

Hormonal measurements
Stress significantly increased the corticosterone level (Fig. 6; main effect of stress: $F_{1,30} = 27.14, P < 0.0001$), and Mgat5−/− mice (stressed, n = 10, 180.72 ± 33.61 ng/ml vs. nonstressed, n = 8, 13.78 ± 7.03 ng/ml) were not significantly different from Mgat5+/+ mice (stressed, n = 7, 192.93 ± 42.03 ng/ml vs. nonstressed, n = 7, 17.76 ± 16.03 ng/ml) (main effect of genotype: $F_{1,30} = 0.06, P > 0.1$).

Discussion
Mgat5−/− pups developed normally and showed no gross impairment in the sensorimotor system, but both sexes showed robust decrease in the immobility duration in tests of depression, the FST and the TST, which can be interpreted as an alteration of depression-like behavior (Berton & Nestler et al. 2008).
2006; Cryan & Holmes 2005). This effect was independent of weight differences, prior behavioral testing, motor activity and changes in anxiety level. Mgat5−/− mice did not respond to antidepressant (desipramine) treatment, whereas desipramine decreased inactive hanging in wild-type and heterozygous subjects (data not shown). One explanation could be the ‘ceiling effect’ of high baseline activity in Mgat5−/− mice, which might prevent detection of further antidepressant effects. Mgat5−/− mice are resistant to weight gain on a calorie-enriched diet, hypersensitive to fasting and display lower respiration quotients and fecundity (Lau et al. 2007). However, fasting did not increase immobility in the FST in Mgat5−/− or Mgat5+/− mice, indicating that acute changes in glucose cannot account for the observed changes in the FST and the TST, but chronic mild hypoglycemia may produce adaptive changes in behavior. Other studies have reported poor replicability for the CMS test (Cryan et al. 2002) and partial resistance of C57BL/6 mice to CMS-induced depression (Ducottet & Belzung 2004; 2005). When Mgat5−/− mice were tested for depression-like behavior subsequent to exposure to the CMS paradigm, they further decreased immobility in the FST and TST, which was significant when the direction of changes (Δnormal) was taken into account. Malfunction of HPA axis is associated with some subtypes of depression and may contribute to chemical imbalance and disease [reviewed in Manji et al. (2001)], but Mgat5−/− mice showed normal corticosterone surge in response to acute stress. However, quantitative trait locus analysis in rats showed no correlation between cortisol levels and FST behavior (Solberg et al. 2003).

Social behavior was also altered in male Mgat5−/− mice estimated in a neutral arena, in which an unfamiliar opponent was used to provide a social stimulus. In the aversive circumstances of the unfamiliar cage, Mgat5−/− mice showed decreased sniffing (P < 0.06) and digging (P = 0.06) when tested in the neutral cage. Mgat5−/−, n = 8; Mgat5+/+, n = 6. (*P < 0.05, **P < 0.01).
could reflect hyperactivity seen under unfamiliar conditions, which has been interpreted in terms of the mania state of bipolar disorder as modeled in animals (Nestler et al. 2002). Hence, it is possible that Mgat5 is linked not only to depression but also mania, which lies at the end of the bipolar affective disorder. The observations of social interactions in the neutral arena support the active phenotype of Mgat5\(^{-/-}\) observed in FST/TST. However, additional experiments are needed to implicate Mgat5 in bipolar disorder (Berton & Nestler 2006; Cryan & Holmes 2005).

The startle reactivity and the average PPI response were not different by genotype for either gender. However, startle reactivity was lower in females compared with males, as previously explained by Plappert et al. (2005).

In brief, our data indicate that in the absence of Mgat5-dependent N-glycans do not cause robust changes in normally low-stress conditions, but do play a pivotal role in the adaptive–maladaptive responses to acute and chronic highly stressful events (i.e. FST, TST and CMS), which leads to depression. A depression-like phenotype can be considered an adaptive response to conditions preserved as inescapably stress (Keller & Nesse 2005; Nesse 2000), where the organism attempts to disengage from useless activity and conserve resources. Mgat5 gene expression is regulated by growth stimulation (Morgan et al. 2004), and the N-glycan processing pathway is also conditionally regulated by metabolite supply to sugar–nucleotide pools. In this regard, decreased immobility in Mgat5\(^{-/-}\) mice may be indicative of failure to adapt with inescapable swimming or hanging, which becomes more prominent after continuation of the stressful conditions when differences in the immobility durations in the two genotypes become more robust. This is consistent with cellular studies in which Mgat5\(^{-/-}\) cells in culture are less responsive to multiple cytokines and to adaptation to changes in glucose levels (Cheung et al. submitted).

Figure 6: Hormonal response to physical restraint. Stress increased the serum corticosterone level (\(P < 0.0001\)), and this response was not different between the genotypes. Striped bars represent restrained mice values, and solid bars denote non-stressed values. Stressed Mgat5\(^{-/-}\), \(n = 10\); stressed Mgat5\(^{+/+}\), \(n = 8\); nonstressed Mgat5\(^{-/-}\), \(n = 7\) and nonstressed Mgat5\(^{+/+}\), \(n = 6\) (** \(P < 0.01\)).

References


Mgat-5 mutants show altered depression-like behavior


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