Semaphorins have an important role in synapse refinement in the mammalian nervous system. The class 3 semaphorin-3F (Sema3F) acting through neuropilin 2/plexin-A3 (Nrp2/PlexA3) holoreceptor complex signals in vivo to restrain apical dendritic spine morphogenesis of cortical pyramidal neurons and hippocampal neurons during postnatal development and mediates excitatory synaptic transmission. Semaphorin signaling has been implicated in the etiology of a number of neurodevelopmental disorders; however, the effects on behavior and mental function of dysregulated Sema3F-Nrp2 signaling have not been fully addressed. The present study is the first behavioral investigation of mice harboring a mutation of the nrp2 gene. Given that loss of Nrp2 signaling alters cortical and hippocampal synaptic organization, we investigated performance of nrp2-deficient mice on learning and sensorimotor function that are known to depend on cortical and hippocampal circuitry. When compared with age-matched controls, nrp2 null mice showed striking impairments in object recognition memory and preference for social novelty. In addition, nrp2−/− mice displayed impaired motor function in the rotarod test and in observations of grooming behavior. Exploration of novel olfactory sensory stimuli and nociception were unaffected by the loss of Nrp2. Overall, loss of Nrp2 may induce aberrant processing within hippocampal and corticostriatal networks that may contribute to neurodevelopmental disease mechanisms.

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INTRODUCTION
Understanding how neurodevelopmental mechanisms establish and organize synaptic connections and ultimately impact mental function may lead to new treatments for a range of neurological and mental disorders.1−5 One important signaling system for navigating growing axons and their growth cones to form the appropriate connections during early nervous system,6 and also regulating postnatal synapse refinement7 involves the large, conserved protein family of the semaphorins. The semaphorin family is composed of at least 25 members across eight subclasses according to structural homology. Most of our knowledge regarding the biological roles of semaphorins in vertebrates comes from studies of the class 3 secreted semaphorins (Sema3s) in the nervous system. However, semaphorins have important roles in a wide range of physiological processes.8−14

The obligate binding partner for most Sema3s, neuropilins were first identified as a neuronal cell surface protein in the Xenopus visual centers,14 and later as a cell adhesion molecule in the chick and mouse nervous system15,16 and was thought to be involved in neuronal recognition of nerve fibers and their targets. It was demonstrated that the two members of the neuropilin (Nrp) family are the receptors for Sema3s; Nrp1 binds with high affinity to Sema3A, whereas Nrp2 preferentially binds to Sema3F.17−20 Due to its short cytoplasmic tail, Nprs were found to be dispensable for signaling in controlling axon guidance and members of the type A plexin (Plex) family was identified as the signal transducing receptor for semaphorin-mediated axon guidance events during development.21−24

Sema3F was shown to signal through Nrp2/PlexA3 receptors to serve as a strong axonal repellent and a pruning factor for hippocampal axons in the developing nervous system.25−27 Indeed, Sema3F have been demonstrated, both in vitro and in vivo, to preferentially signal through the Nrp2/PlexA3 holo-receptor complex,19,20,28 as defects observed in both embryonic and postnatal development are recapitulated from mice deficient in the Sema3F−Nrp2/PlexA3 signaling pathway.25,29−31 Previously, we and others have shown that Sema3F−Nrp2/PlexA3 signals in vivo to restrain apical dendritic spine morphogenesis in layer 5 cortical pyramidal neurons during postnatal development and mediate excitatory synaptic transmission.32,33 Nrp2 expression is enriched in the molecular layer of the hippocampal formation, where the dentate gyrus granule cell dendrites reside. Sema3F is robustly expressed in the hilus, along the projection pathways of both the supra and intrapyramidal axons and the entorhinal cortex axon that innervate the dentrites of the molecular layer.25 Indeed, both Sema3F and Nrp2 mutants displayed an increase in dendritic spine number, distribution, size and miniature excitatory postsynaptic current frequency in both hippocampal dentate granule cells and layer 5 cortical neurons.33 Therefore, Seam3F and Nrp2 expression pattern and function in the postnatal brain is consistent with the hypothesis that these proteins direct cortical and hippocampal neural circuit formation.

Semaphorin dysregulation has been linked to a range of neurological disorders,34−37 and may have a key role in learning and memory by modulating synaptic plasticity in the adult hippocampus.32,38 Nevertheless, the effects on behavior of dysregulated Sema3F/Nrp2 signaling remain unknown. Here, we tested mice on a range of tasks, including those that depend on hippocampal and corticostriatal circuits that we have shown to be altered by nrp2 knockout. Dysregulation of these circuits have

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been implicated in a number of mental disorders including autism and schizophrenia; therefore, understanding their function is of particular relevance to understanding disease mechanisms. Specifically, we used recognition memory tasks that depend on the dentate gyrus and its projections into the CA3 subregion of the hippocampus. This circuit has a key role in pattern separation—the process of transforming similar representations or memories into highly dissimilar, nonoverlapping representations. 29-41 In addition, we examined the acquisition of a repetitive motor behavior that relies on corticostriatal circuitry. 42-44 We show that Nrp2-deficient animals are impaired in object and social recognition memory and repetitive motor behavior, but display normal sensory processing. Taken together, our results reveal novel functions of Sema3F–Nrp2 signaling in complex behavior output.

MATERIALS AND METHODS

Mice

The Nrp2 knockout mice have been previously described in detail, both its expression patterns and developmental phenotypes. 29 Mice used in this study have been backcrossed for 10 plus generations to the C57BL/6NTac background strain, and only males (n = 5–7 per group) were used for all behavioral testing. We used wild-type (+/+) littermates as controls, heterozygous (+/−), and homozygous (−/−) nrp2 mice. However, heterozygous nrp2 mice display a normal neural anatomical and electrophysiological phenotype. 59,63,44-46 In addition, we observed in our mouse-breeding colony that the nrp2 locus does not follow the Mendelian 3:1 ratio of distribution; in fact, the ratio of inheritance for the homozygous mutant is much less. Thus to circumvent this hurdle, we have optimized a 3:1 ratio of distribution; in fact, the ratio of inheritance for the homozygous mutant is much less. Thus to circumvent this hurdle, we have optimized 47 In addition, we observed in our mouse-breeding colony that the nrp2 locus does not follow the Mendelian 3:1 ratio of distribution; in fact, the ratio of inheritance for the homozygous mutant is much less. Thus to circumvent this hurdle, we have optimized 47 In addition, we observed in our mouse-breeding colony that the nrp2 locus does not follow the Mendelian 3:1 ratio of distribution; in fact, the ratio of inheritance for the homozygous mutant is much less. Thus to circumvent this hurdle, we have optimized the process of transforming similar representations or memories into highly dissimilar, nonoverlapping representations. 29-41 In addition, we examined the acquisition of a repetitive motor behavior that relies on corticostriatal circuitry. 42-44 We show that Nrp2-deficient animals are impaired in object and social recognition memory and repetitive motor behavior, but display normal sensory processing. Taken together, our results reveal novel functions of Sema3F–Nrp2 signaling in complex behavior output.

Novel object recognition test. Novel object recognition testing was based on previously described procedures. 29 Mice were tested with two objects in a 40 cm×40 cm (w/d) open-field arena. During the sample phase, two identical objects were placed in opposite corners of the arena 10 cm from the nearest walls. Mice were placed in the center of the arena and allowed to freely investigate both objects for 10 min after which they were returned to their home cage for 30 min. During the 5-min test phase, mice encountered one ‘familiar’ object from the sample phase and a novel object. The number of sniffs to the familiar and novel object was assessed and quantified from video footage by observers that were blind to the genotype of the animals. The objects consisted of a white plastic funnel and a white and yellow bird-shaped toy and were wiped down with isopropyl alcohol between phases.

Social novelty test. Preference for social novelty was tested in a three-chambered arena, modified from that previously described. 49 Each of the three chambers of the arena were equally sized and separated from each other by a plexi-glass barrier. A small hole allowed passage between the chambers. Mice were first habituated to the empty arena for 30 min. During the 10-min sample phase, an unfamiliar male mouse was confined to one of the chambers by a small wire cage placed over it and the test mouse was allowed to freely roam the apparatus for 10 min. The opposite chamber contained a wire cage with no mouse. During the test phase, the mouse from the sample phase was returned to the apparatus and confined to one chamber. This mouse was designated the ‘familiar’ mouse. An unfamiliar mouse was confined to the opposite chamber. The location of the familiar and novel mice was randomized across subjects. The test mouse was placed in the center of the arena and allowed to freely roam the apparatus for 10 min. The amount of time spent in each chamber was assessed and quantified using tracking analysis from Noldus Observer software (Noldus, Leesburg, VA, USA).

Rotarod test. Mice were tested on a standard rotarod device (Med Associates, St Albans, VT, USA). Mice were placed on the spindle, which linearly accelerated from 4 to 40 r.p.m. The trial ended when the mouse fell off the spindle, made one complete revolution while gripping the spindle, or 5 min had elapsed. Each mouse received five trials per day over 2 days with ~10 min between trials.

Hot-plate test. Mice were placed in a standard hot-plate apparatus. The hot plate measured 10 cm×10 cm and was surrounded by a plastic enclosure. Mice were initially habituated to the apparatus for 5 min. During the test, the temperature was set to 55 °C. Mice were placed in the center of the apparatus for 2 min. Videotape footage was scored by two trained observers, who were blind to the genotype of the animals. The number of hind limb withdrawals was recorded and quantified.

Grooming, rearing and bed-pushing behavior. Mice were videotaped for 20 min in a cage filled with cedar bedding that was identical to their home cage. Two trained observers, who were blind to the genotype of the animals, scored and quantified the frequency of rearing and grooming events and displacement of bedding material over the 20-min time period.

Olfactory habituation/dishabituation. Mice were sequentially presented with scented cotton swabs and investigatory behavior was quantified from video footage by observers that were blind to the genotype of the animals. Each cotton swab was inserted into the cage for 2 min and affixed to the cage lid. Sniffs were counted and quantified when the nose was oriented toward and within 2 cm of the swab. Any bouts that involved physical contact with the swab was not counted. Mice received three consecutive presentations of swabs soaked in distilled water, followed by three presentations of swabs soaked in vanilla or banana extract that was diluted to 1:100 concentration. Mice then received presentations of swabs wiped in the bedding of conspecific male mice.

Data analysis

Data analysis was conducted in SPSS (IBM, Chicago, IL, USA). Data from behavioral observations were averaged from two observers and subjected to analyses of variance (ANOVAs) and t-tests with Bonferroni post hoc correction for multiple comparisons. Data from the novel object and social novelty tests were converted into ratios (‘familiar’ and ‘novel’ as a percent of total behavior). These data were subjected to two-factor ANOVAs with genotype as a between-subjects factor and novelty as a within-subject factor.

RESULTS

Nrp2 knockout alters novel object recognition and social novelty behavior

We hypothesized that nrp2−/− mice would show a deficit in tasks that depend on hippocampal function, particularly those mediated by the dentate gyrus mossy fiber pathway. 29-41 To test this hypothesis, we evaluated performance in novel object and social novelty tests that are known to rely on the functions of these subregions of the hippocampus. 50,51 Counting the number of sniffs directed at novel and familiar objects during the recognition test, we found a significant preference to investigate novel objects among wild-type and nrp2−/− mice. Data were normalized by dividing the number of sniffs for each object by the total number of sniffs during the test. The normalized data depicted in Figure 1a shows a preference for novelty among wild-type (68% novel object; 32% familiar object) and nrp2−/− mice (68% novel object; 32% familiar object normalized number of sniffs per object). Group comparisons using paired-samples t-tests revealed that preference for novelty was significant for both groups (wild-type t5 = 3.42; nrp2−/−, t5 = 3.47, P < 0.05) (Figure 1a). In contrast, no difference in investigatory behavior among novel and familiar objects was observed among nrp2−/− mice (51% novel object; 49% familiar object normalized number of sniffs per object). An analysis of the total number of investigatory behaviors directed at the two objects revealed no effect of genotype on sniffing frequency (Figure 1b). Wild-type, heterozygous and null mice made similar amounts of object-directed sniffs during the test (average sniffs per minute: wild-type 9.1 novel object, 4.6 familiar object; nrp2−/−...
We found no effects on bed-pushing behavior (Figure 4a). In addition, an analysis of locomotion (total distance traveled) during the first test phase found no effects between genotypes (ANOVA $P=0.67$; Figure 1b-inset). Overall these data reveal that all mouse strains were equally engaged in the task, but the $nrp2$ knockout strain alone failed to show a significant object preference.

Next, we asked whether Nrp2 signaling influences social recognition memory. Recording the amount of time spent in the chamber containing a novel or familiar mouse during the preference test, we found a significant preference for novelty among wild-type and $nrp2^{+/−}$ mice. Data were normalized by dividing the amount of time spent in each chamber by the total amount of time spent in both chambers. The normalized data depicted in Figure 2 (and also see Supplementary Video) show a significant preference for novelty among wild-type (79% novel mouse; 21% familiar mouse normalized time spent in each chamber) and $nrp2^{−/−}$ (69% novel mouse; 31% familiar mouse normalized time spent in each chamber) mice. Group comparisons using paired-samples t-tests revealed significant differences in novelty preferences for both the groups (wild-type $t_8=7.32; \text{nrp}^{−/−} t_4=6.49, P < 0.01$). No preference was observed in $nrp2^{−/−}$ mice (51% novel mouse; 49% familiar mouse normalized time spent in each chamber). The lack of preference for a novel mouse among knockout mice was not due to a lack of sociability. During the first familiarization phase of the test, $nrp2^{−/−}$ mice spent significantly longer time with a mouse compared with a chamber containing an empty enclosure ($t_6=6.48, P < 0.01$, data not shown).

Nrp2 knockout impairs repetitive motor behavior but preserves sensory processing

Nrp2 knockout alters cortical layer V neurons, which form the primary cortical input to the striatum. We hypothesized that tasks, such as the rotarod, that rely on corticostriatal circuitry would be impaired in $nrp2^{−/−}$ mice. We acknowledge that while it is customary to use wild type as controls, we believe it is justifiable to use heterozygous here as controls for the following reasons. On the basis of previous published neural developmental results for Nrp2 signaling, all characterized phenotypes observed are recessive and heterozygous animals display normal neural anatomical and electrophysiological phenotypes identical to the wild-type littermates. Thus, we believe that in most cases the heterozygous is a better control group as they are most similar genetically to the homozygous null, but do not show the neural defects reported in the null animals.

We found a significant impairment in rotarod performance among $nrp2$ mice, with the latency to fall significantly greater among the knockout strain compared with their control heterozygous counterparts (ANOVA $F_{1,10}=4.78, P = 0.05$, Figure 3). The latency to fall was significantly shorter in $nrp2^{+/−}$ compared with controls ($+/−$) beginning at trial 8. To further examine motor behavior, we made detailed observations of home-cage behavior. We found no effects on bed-pushing behavior (Figure 4a). However, we found that $nrp2^{−/−}$ mice made significantly longer grooming bouts compared with wild-type ($P = 0.03$) and $nrp2^{+/−}$ mice ($P = 0.02$, Figure 4b). In addition, knockout mice made significantly more frequent grooming bouts ($P < 0.01$) and rearing
behavior ($P = 0.03$) compared with wild-type mice (Figures 4b and d). Overall, these data suggest that acquisition or performance of repetitive motor behavior in $\text{nrp2}^{-/-}$ mice is impaired by lack of Nrp2 function.

To examine the possibility of sensory dysfunction caused by $\text{nrp2}$ knockout, we tested mice on a hot-plate test and an olfactory habituation/dishabituation task. We found no effects of genotype on hot-plate performance. The frequency of hind-foot movements during exposure to the hot plate did not differ among genotypes ($P > 0.87$, Figure 5a). Similarly, we found no effect of $\text{nrp2}$ knockout in olfactory investigatory behavior. Habituation to sensory stimuli does not require hippocampal engagement when stimuli are sequentially presented without delay, therefore performance in this task primarily assesses sensory function. All mice showed investigatory behavior directed toward odor stimuli that was enhanced by novelty. Mice, independent of genotype, sniffed more frequently to a novel odor compared with a familiar odor as shown by a main effect of odor novelty on snifffing frequency independent of other factors (repeated-measures ANOVA $F_{1,14} = 6.62$, $P = 0.02$, Figure 5b). Although there was no effect of genotype on sniffing frequency, there were significant interactions involving genotype and odor type (social versus nonsocial, ANOVA $F_{2,14} = 4.84$, $P = 0.02$). Heterozygous and knockout mice made significantly more sniffs to nonsocial odors compared with wild-type mice, whereas no difference in sniffing frequency among genotypes was observed for social odors. Overall, these data indicate intact olfactory sensory processing in $\text{nrp2}^{-/-}$ mice. Therefore, the deficits in recognition memory that were observed are not likely a consequence of impaired sensory function.

**DISCUSSION**

We believe our findings are the first detailed behavioral investigation of $\text{nrp2}$-deficient mice. Given that loss of Nrp2 signaling alters
hippocampal and cortical synaptic organization, we hypothesized that these mice would display deficits in tasks that depend on these circuits. In support of this hypothesis, \( \text{npr}^2{-/-} \) mice were significantly impaired in tests of object and social recognition memory. We additionally found impaired rotarod performance, suggesting that \( \text{npr}^2 \) knockout impacts the acquisition or performance of repetitive motor behavior. Overall, we found that Sema3F-Nrp2 signaling fundamentally influences behavior, either through their actions on neural circuit formation during embryonic brain development or postnatal synaptic refinement and plasticity.

\( \text{npr}^2{-/-} \) mice were impaired in tests of novel object recognition and social novelty. Both tests rely on the animal acquiring and retaining an episodic-like memory of the sensory features of an object or mouse and using this information during the test phase to guide their investigatory behavior. Performance of \( \text{npr}^2{-/-} \) mice in both tests may therefore reflect impaired acquisition or retention of long-lasting episodic-like memory. Accurate performance on these tasks is known to depend on the hippocampal formation.\(^{50,51}\) Previously, we have shown altered synapse structure and physiology in the hippocampus of \( \text{npr}^2{-/-} \) mice.\(^{33}\) Therefore, impaired performance that was observed in \( \text{npr}^2{-/-} \) mice on these tasks may represent a defect in synaptic plasticity in the hippocampus. Further investigation using complex mouse genetics such as conditional knockout animals crossed to specific CreER-lines, which is beyond the scope of this study, will determine whether the deficits in behavior that were observed are due to the effects of loss of function in embryonic, postnatal or adult animals.

Although our study is not a comprehensive behavioral phenotype and the possibility remains that factors such as anxiety or impaired sensory processing could influence performance on the novelty tests, additional evidence we gathered would argue against this possibility. For example, we found no significant difference between \( \text{npr}^2{-/-} \) mice and matched controls in exploratory activity during the learning phases of these tests. If anxiety prevented these mice from learning then presumably it would have significantly altered behavior during the learning phase. Likewise, we found no differences between strains in bedpushing behavior, an indicator of anxiety in mice. We found no impairment in responses to nociceptive or olfactory stimuli suggesting intact sensory processing in \( \text{npr}^2{-/-} \) mice. Another possible explanation for our findings is that \( \text{npr}^2{-/-} \) mice may have intact hippocampal processing but show no novelty preference. This interpretation is unlikely given that we found \( \text{npr}^2{-/-} \) mice investigated objects and mice during the learning phase and during the olfactory habituation task, suggesting an intact interest in these animals in exploring novel environmental stimuli.

We found impairments in repetitive motor behavior in \( \text{npr}^2{-/-} \) mice. Mice were impaired in rotarod performance and made longer grooming bouts. \( \text{npr}^2 \) deletion increases excitability and spine density in layer 5 cortical pyramidal neurons, which form the cortical projection to the basal ganglia. Corticobasal ganglia circuitry is responsible for action control and alterations to this circuit can increase motor stereotypy.\(^{52,53}\) Therefore, the present findings may be explained in the context of impaired corticobasal ganglia function. However, additional experiments beyond the scope of this study will be necessary to fully test this hypothesis.

Our results are consistent with other behavioral studies in animals deficient in semaphorin signaling. For example, Sema6A-deficient animals possess alterations in cortical and limbic organization and show impaired object recognition memory and hyper-exploratory behavior,\(^{37}\) similar to the pattern of behavior that was observed in \( \text{npr}^2{-/-} \) mice, whereas loss of Sema4D enhances motor behavior.\(^{54} \) More broadly, our findings are consistent with behavioral deficits observed in mice with neurodevelopmental defects affecting synaptic organization.\(^{55,56}\) Overall, our results provide novel evidence of behavioral modification in Sema3F-Nrp2 signaling. \( \text{npr}^2 \) null mice showed striking impairments in both hippocampal-dependent memory tasks and repetitive motor behavior. The changes in behavior that were observed may reflect aberrant processing within hippocampal and cortical networks known to be impacted by \( \text{npr}^2 \) knockout. Finally, our findings are relevant to understanding neurodevelopmental diseases such as ASD: the behavioral phenotype matches some of the core features of ASD (learning impairments, repetitive behavior) and the neural phenotype of altered cortical microcircuitry is consistent with contemporary theories of ASD pathophysiology.\(^{57,58}\)

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

MWS and TST designed the study, analyzed the data and wrote the manuscript. MG carried out the majority of the experiments and MWS assisted in some of the behavioral testing procedures.

REFERENCES

1 Geschwind DH. Autism: many genes, common pathways? Cell 2008; 135: 391–395.
2 Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. Curr Opin Neurobiol 2007; 17: 103–111.
3 Castro J, Mellios N, Sur M. Mechanisms and therapeutic challenges in autism spectrum disorders: insights from Rett syndrome. Curr Opin Neurobiol 2013; 26: 154–159.
4 Brandon NJ, Sawa A. Linking neurodevelopmental and synaptic theories of mental illness through DISC1. Nat Rev Neurosci 2011; 12: 707–722.
5 Qiu S, Aldinger KA, Levitt P. Modeling of autism genetic variations in mice: focusing on synaptic and microcircuit dysfunctions. Dev Neurosci 2012; 34: 88–100.
6 Kolodkin AL, Tessier-Lavigne M. Mechanisms and molecules of neuronal wiring: a primer. Cold Spring Harb Perspect Biol 2011; 3: a001727.
7 Koropouli E, Kolodkin AL. Semaphorins and the dynamic regulation of synapse assembly, refinement, and function. Curr Opin Neurobiol 2014; 24: 1–7.
8 Tran TS, Kolodkin AL, Bharadwaj R. Semaphorin regulation of cellular morphology. Annu Rev Cell Dev Biol 2007; 23: 263–292.
9 Pasterkamp RJ. Getting neural circuits into shape with semaphorins. Dev Neurosci 2013; 35: 371–378.

6 Bagi A, Cheng HJ, Yaron A, Pleasure SJ, Tessier-Lavigne M. Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. Cell 2003; 113: 285–299.

21 Takagi S, Kasuya Y, Shimizu M, Matsuura T, Tsuboi M, Kawakami A. Mutation of Semaphorin-6A disrupts limbic and cortical connectivity and models neurodevelopmental psychopathology. PLoS One 2011; 6: e26488.

8 Lee K, Kim JH, Kwon OB, An K, Ryu J, Cho K et al. An activity-regulated microRNA, mir-188, controls dendritic plasticity and synaptic transmission by down-regulating neuropilin-2. J Neurosci 2012; 32: 5678–5687.

23 Takahashi T, Fournier A, Zou Y, Sneath S, Pleasure SJ, et al. Neuruplin regulates the development of selective cranial and sensory nerves and hippocampal mossy fiber projections. Neuron 2003; 25: 43–56.

154 Yaron A, Huang PH, Cheng HJ, Tessier-Lavigne M. Differential requirement for Plexin-A3 and -A4 in mediating responses of sensory and sympathetic neurons to distinct class 3 Semaphorins. Neuron 2005; 45: 513–523.

26 Bagi A, Cheng HJ, Yaron A, Pleasure SJ, Tessier-Lavigne M. Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. Cell 2003; 113: 285–299.

27 Riccomagno MM, Hurtado A, Wang H, Macopson JG, Grimer EM, Betz A et al. The RacGAP beta2-Chimaerin selectively mediates axonal pruning in the hippocampus. Cell 2012; 149: 1594–1606.

28 Murakami Y, Suto F, Shimizu M, Shinoh D, Kameyama T, Fujisawa H. Differential expression of plexin-A subfamily members in the mouse nervous system. Dev Dyn 2001; 220: 246–258.

29 Giger RJ, Clouter JF, Sahay A, Pinjha RK, Levegndov DV, Moore SE et al. Neuroplin-2 is required in vivo for selective axon guidance responses to secreted semaphorins. Neuron 2000; 25: 29–41.

30 Chen H, Bagi A, Zupicich JA, Zou Y, Sneath S, Pleasure SJ et al. Neuropilin-2 regulates the development of selective cranial and sensory nerves and hippocampal mossy fiber projections. Neuron 2003; 25: 43–56.

31 Yaron A, Huang PH, Cheng HJ, Tessier-Lavigne M. Differential requirement for Plexin-A3 and -A4 in mediating responses of sensory and sympathetic neurons to distinct class 3 Semaphorins. Neuron 2005; 45: 513–523.

32 Sahay A, Kim CH, Seokpy J, Cho E, Huganir RL, Ginty DD et al. Secreted semaphorins mediate synaptic transmission in the adult hippocampus. J Neurosci 2005; 25: 3631–3620.

33 Tran TS, Rubio ME, Clemen RL, Johnson D, Case L, Tessier-Lavigne M et al. Secreted semaphorins control spine distribution and morphogenesis in the postnatal CNS. Nature 2009; 462: 1065–1069.

34 Wu S, Yue W, Jia M, Ruan Y, Lu T, Gong X et al. Association of the neuropilin-2 (NRPRP) gene polymorphisms with autism in Chinese Han population. Am J Med Genet B Neuropsychiatr Genet 2007; 144B: 492–495.

35 Gint J, Thibault O, Blalock E, Yang J, Bachstetter A, Kotick J et al. Decreased number of interneurons and increased seizures in neuropilin 2 deficient mice: implications for autism and epilepsy. Epilepsia 2009; 50: 629–645.

36 Brooks-Kayal A. Epilepsy and autism spectrum disorders: are there common developmental mechanisms? Brain Dev 2010; 32: 731–738.

37 Runker AE, O'Tuathaigh C, Dunleavy M, Morris DW, Little GE, Corvin AP et al. Mutation of Semaphorin-6A disrupts limbic and cortical connectivity and models neurodevelopmental psychopathology. PLoS One 2011; 6: e26488.

38 Lee K, Kim JH, Kwon OB, An K, Ryu J, Cho K et al. An activity-regulated microRNA, mir-188, controls dendritic plasticity and synaptic transmission by down-regulating neuropilin-2. J Neurosci 2012; 32: 5678–5687.

39 Bakker A, Kirvan CB, Miller M, Stark CE. Pattern separation in the human hippocampus CA3 and dentate gyrus. Science 2008; 319: 1640–1642.

40 Deng W, Mayford M, Gage FH. Selection of distinct populations of dentate granule cells in response to inputs as a mechanism for pattern separation in mice. Elife 2013; 2: e00312.

41 Rolli ET. The mechanisms for pattern completion and pattern separation in the hippocampus. Front Syst Neurosci 2013; 7: 74.

42 Costa RM, Cohen D, Nicolelis MA. Differential corticostriatal plasticity during fast and slow motor skill learning in mice. Curr Biol 2004; 14: 1124–1134.

43 Doyon J, Penhune V, Ungereider LG. Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. Neuropsychologia 2003; 41: 252–262.

44 Yin HH, Mulcare SP, Hilario MR, Clouse E, Holloway T, Davis MI et al. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nat Neurosci 2009; 12: 333–341.

45 Clouter JF, Giger RJ, Koenigts GB, Daluc C, Kolodkin AL, Ginty DD. Neuroplin-2 mediates axonal fasciculation, zonal segregation, but not axonal convergence, of primary accessory olfactory neurons. Neuron 2002; 33: 877–892.

46 Clouter JF, Sahay A, Chang EC, Tessier-Lavigne M, Daluc C, Kolodkin AL et al. Differential requirements for semaphorin 3 F and Slt-1 in axonal targeting, fasciculation, and segregation of olfactory sensory neuron projections. J Neurosci 2004; 24: 9087–9096.

47 Huber AB, Kania A, Tran TS, Gu C, De Marco Garcia N, Lieberam I et al. Distinct roles for secreted semaphorin signaling in spinal motor axon guidance. Neuron 2007; 54: 949–964.

48 Bevins RA, Besheer J. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study ‘recognition memory’. Nat Protoc 2006; 1: 1306–1311.

49 Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. Nat Rev Neurosci 2010; 11: 490–502.

50 Cohen SJ, Munchow AH, Rios LM, Zhang G, Agelesdinotis HH, Stackman RW Jr. The rodent hippocampus is essential for nonspatial object memory. Curr Biol 2013; 23: 1685–1690.

51 Broadbent NJ, Gaskin S, Squire LR, Clark RE. Object recognition memory and the rodent hippocampus. Learn Mem 2010; 17: 5–11.

52 Singer HS. Motor control, habits, complex motor stereotypes, and Tourette syndrome. Ann N Y Acad Sci 2013; 1304: 22–31.
53 Muehlmann AM, Lewis MH. Abnormal repetitive behaviours: shared phenomenology and pathophysiology. J Intellect Disabil Res 2012; 56: 427–440.

54 Yukawa K, Tanaka T, Takeuchi N, Iso H, Li L, Kohsaka A et al. Sema4D/CD100 deficiency leads to superior performance in mouse motor behavior. Can J Neurol Sci 2009; 36: 349–355.

55 Cheh MA, Millonig JH, Roselli LM, Ming X, Jacobsen E, Kamdar S et al. En2 knockout mice display neurobehavioral and neurochemical alterations relevant to autism spectrum disorder. Brain Res 2006; 1116: 166–176.

56 Briemlaier J, Matteson P, Silverman J, Senerth J, Kelly S, Genestine M et al. Autism-relevant social abnormalities and cognitive deficits in engrailed-2 knockout mice. PLoS One 2012; 7: e40914.

57 Markram H, Rinaldi T, Markram K. The intense world syndrome—an alternative hypothesis for autism. Front Neurosci 2007; 1: 77–96.

58 Rinaldi T, Silberberg G, Markram H. Hyperconnectivity of local neocortical microcircuitry induced by prenatal exposure to valproic acid. Cereb Cortex 2008; 18: 763–770.

Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)