Complexin 1 knockout mice exhibit marked deficits in social behaviours but appear to be cognitively normal

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Complexins are presynaptic proteins that modulate neurotransmitter release. Abnormal expression of complexin 1 (Cplx1) is seen in several neurodegenerative and psychiatric disorders in which disturbed social behaviour is commonplace. These include Parkinson’s disease, Alzheimer’s disease, schizophrenia, major depressive illness and bipolar disorder. We wondered whether changes in Cplx1 expression contribute to the psychiatric components of the diseases in which Cplx1 is dysregulated. To investigate this, we examined the cognitive and social behaviours of complexin 1 knockout mice (Cplx1<sup>−/−</sup>) mice. Cplx1<sup>−/−</sup> mice have a profound ataxia that limits their ability to perform co-ordinated motor tasks. Nevertheless, when we taught juvenile Cplx1<sup>−/−</sup> mice to swim, they showed no evidence of cognitive impairment in the two-choice swim tank. In contrast, although olfactory discrimination in Cplx1<sup>−/−</sup> mice was normal, Cplx1<sup>−/−</sup> mice failed in the social transmission of food preference task, another cognitive paradigm. This was due to abnormal social interactions rather than cognitive impairments, increased anxiety or neophobia. When we tested social behaviour directly, Cplx1<sup>−/−</sup> mice failed to demonstrate a preference for social novelty. Further, in a resident–intruder paradigm, male Cplx1<sup>−/−</sup> mice failed to show the aggressive behaviour that is typical of wild-type males towards an intruder mouse. Together our results show that in addition to the severe motor and exploratory deficits already described, Cplx1<sup>−/−</sup> mice have pronounced deficits in social behaviours. Abnormalities in complexin 1 levels in the brain may therefore contribute to the psycho-social aspects of human diseases in which this protein is dysregulated.

INTRODUCTION

Complexins are small presynaptic proteins that bind to the soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) assembly (1,2). They stabilize the SNARE complex in a docked and primed state ready for fast calcium-mediated exocytosis (3–5). Current evidence suggests that complexins inhibit full vesicle fusion, and their binding sustains the vesicle in a partially fused state (6).

There are two main isoforms of complexin in wild-type mouse brain (7–9), complexin I (Cplx I) and complexin II (Cplx II). These are expressed throughout the mouse brain in a generally reciprocal manner. Cplx I is expressed in the cerebellum, thalamus, CA3 of the hippocampus and layers IV and V of the cortex, whereas Cplx II is expressed in the striatum, CA1 of the hippocampus, the amygdala and layers II/III and V/VI of the cortex (7–9). We have postulated previously that Cplx I plays a key role in motor learning, involuntary movement and sensory processing and that Cplx II plays a role in cognitive function and the control of voluntary movement and emotion (8). In support of this, we have shown that complexin 2 knockout (Cplx2<sup>−/−</sup>) mice exhibit progressive motor and cognitive deficits (10) and that complexin 1 knockout (Cplx1<sup>−/−</sup>) mice have a severe motor phenotype coupled with exploratory and habituation deficits (11).

There is considerable evidence suggesting that dysregulation of complexin expression occurs in neurological disease, in particular, neurodegenerative disorders. There is a selective (compared with other synaptic markers such as synaptophysin, syntaxin and synaptobrevin) downregulation of CPLX2 mRNA in the early stages of Huntington’s disease (HD) in both post-mortem brains and the R6/2 mouse model of HD (12–14). Cplx1 mRNA is also downregulated in R6/2 mice when they are in the later stages of the disease (15).
There is selective decreased expression of Cplx I and Cplx II in the thalamic and inferior colliculus in experimental Wernicke’s encephalopathy (16). Further, selective decreased expression of CPLX II is seen in the hippocampus, and decreased expression of CPLX I is found in the inferior temporal cortex in Alzheimer’s disease (AD) brains (17). Notably, although in most diseases there is a decrease in complexin expression, in Parkinson’s disease (PD), an increase in the expression of CPLX I has been reported in the substantia nigra (18). As well as neurodegenerative diseases, complexin expression is altered in several important psychiatric diseases. Decreases in expression of both complexin isoforms at the mRNA and protein level have been described in schizophrenia (19–22) and bipolar disorder (23,24). A selective decrease of CPLX I has been reported in the pre-frontal cortex in major depressive illness (25), and a selective decrease in both complexin isoforms is seen in rats bred for ‘learned helplessness’, thought to be an animal correlate of depression (26). Together these studies show that complexins are dysregulated in neurological disease and support the idea that there is an optimal amount of complexin in the synapse. That is, having either too much or too little complexin can lead to impairments in neurological function (3,27).

Interestingly, in many of the disorders in which CPLX I and CPLX II expressions are altered, patients exhibit complex symptomology that includes cognitive, motor and psychiatric disturbances. Cognitive disturbance is the major symptom in AD, and cognitive abnormalities are well described in both HD and PD. Further, cognitive as well as motor abnormalities have been described in both R6/2 mice (28,29) and Cplx2−/− mice (10). However, cognitive function has not been examined in Cplx1−/− mice. Here we used four different behavioural paradigms to investigate the cognitive and social phenotypes of Cplx1−/− mice with a view to gaining a better understanding of the role of complexins in neurological disease.

**RESULTS**

**Juvenile Cplx1−/− mice can learn to swim**

We reported recently that mature adult (more than 20 weeks of age) Cplx1+/− mice cannot swim (11). Here we show that juvenile Cplx1−/− mice can be taught to swim at 4 weeks of age. Adult Cplx1−/− mice do not learn to swim because they display a ‘panic’ response when placed in the water (11). Combined with an adult body weight, this makes them sink. Adult Cplx1−/− mice make dystonic movements with all limbs and kick in an uncoordinated manner. This causes them to twist and turn in the water, which also means they cannot orientate themselves correctly and keep their heads above the water. Juvenile mice are lighter than adult mice and can float. We wondered whether they could learn to overcome the panic reaction, and if so whether they could be ‘taught’ to swim. Wild-type mice placed in water exhibit reflex swimming behaviour, which is not seen in Cplx1−/− mice. To teach juvenile Cplx1−/− mice to swim, they were introduced to the swim tank as slowly as possible; being lowered into the water over 4–5 s. This allowed Cplx1−/− mice to keep their body as horizontal to the water as possible, which in turn minimized the panic response usually seen in the older Cplx1−/− mice. At the beginning of swimming training, the swimming style of Cplx1−/− mice was very poor. Whereas Cplx1+/+ mice swim immediately using their hind limbs for propulsion and their front paws tucked up, Cplx1−/− mice kick with all four limbs and displayed dystonic movements. The dystonic limb-kicks unbalanced the Cplx1−/− mice and caused them to swim with a ‘twisting’ motion of the hind quarters. This dystonic swimming style improved slightly with training but Cplx1−/− mice never learnt to swim as proficiently as Cplx1+/+ mice or heterozygote mice (Cplx1−/+).

The dystonic movements of Cplx1−/− mice meant that although the mice quickly learned (within a few trials) to propel themselves in a forward direction, changing direction and turning around was always very difficult for them. This did not improve with training and contributed to their slow performance in this task. Cplx1−/− mice also adapted their swimming strategy in the swim tank by swimming against the walls of the tank using the edge of the tank as support, in much the same way that a poor human swimmer uses the edge of a swimming pool for support. Cplx1−/− mice, but not Cplx1+/+ mice, also used the wall to help them to change direction.

**Cplx1−/− mice show no evidence of cognitive deficits in the two-choice swim tank**

Performance in the two-choice swim tank task does not depend on swim speed or strength for an outcome (28). This is because it is the direction in which the mouse chooses to swim (i.e. directly towards or away from the light) that is assessed as a measure of cognitive function, not the time taken to reach the platform. This made this task an ideal paradigm to investigate the cognitive function of Cplx1−/− mice. Despite the slower swim speed of Cplx1−/− mice, there were no differences in the acquisition of the task between Cplx1−/− mice, Cplx1+/+ mice and Cplx1−/+ mice (Fig. 1A). In fact Cplx1−/− mice actually learned the reversal of the task more quickly than Cplx1+/+ mice or Cplx1−/+ mice (genotype x testing day, $F_{10,310} = 12.56, P < 0.01$).

It is possible that the increased latency was due to an increase in the time taken to make a decision. We have no way of testing this. However, it is more likely to be a consequence of swimming impairments, since the poor swimming style of Cplx1−/− mice was reflected in the time it took them to reach the platform when compared with Cplx1+/+ mice or Cplx1−/+ mice (Fig. 1B, genotype, $F_{1,21} = 47.96, P < 0.001$). Although Cplx1−/− mice took longer to reach the platform, this did not affect their performance in the task, as the two-choice swim tank paradigm measures a correct response by the direction of the swimming, not the swimming speed. The time taken to reach the platform for Cplx1−/− mice improved steadily throughout the test period (testing day, $F_{2,21} = 13.28, P < 0.001$). By the end of the acquisition phase, the latency of Cplx1−/− mice to swim to the platform was not significantly different to that of Cplx1+/+ mice or Cplx1−/+ mice. On the first day of reversal, the time taken to reach the platform increased significantly compared with the time it had taken to reach the platform on the last day of acquisition (genotype x testing day, $F_{1,13} = 49.57, P < 0.001$). This effect was more pronounced
Figure 1. Cplx1−/− mice show no evidence of cognitive impairment in the two choice swim tank. Cplx1−/− mice learned both the acquisition (days 1–8) and reversal (days 10–20) phases of the task as efficiently as Cplx1+/+ and Cplx1+/− mice (A). The difficulty that Cplx1−/− mice had in swimming was evident in the greater latency taken to reach the platform although this improved over the course of the experiment (B). Asterisks indicate significant differences between Cplx1+/+ and Cplx1−/− mice (***P < 0.001, ANOVA). Post hoc analysis revealed significant differences in the latency to the platform on day 1 (P < 0.001), days 2 and 3 (P < 0.01), days 10–13 inclusive (P < 0.001), days 14 and 15 (P < 0.01) and day 16 (P < 0.05). Symbols show the mean ± SEM of % correct choices made and latency to reach the platform. Where error bars are not visible they are obscured by the symbols.

in Cplx1−/− mice than in Cplx1+/+ mice or Cplx1+/− mice. The increase in time taken for Cplx1−/− mice to reach the platform was not due to a learning deficit, but rather was due to the fact that when they swam to the ‘wrong’ end of the tank they had to turn around and swim back past the start area to the platform. This was compounded by the fact that when Cplx1−/− mice took longer than about 15 s to find the platform, their swimming performance deteriorated and the ‘twisting’ motion described earlier worsened. This, combined with slow turning of Cplx1−/− mice, accounted for the increased latency on the first day of reversal. The latency to reach the platform improved during the reversal phase of the task in all mice (testing day, F_{10,310} = 46.39, P < 0.001). By day 17 when all the mice were making a similar number of correct choices, the latency to the platform did not differ significantly between Cplx1+/+ mice, Cplx1−/− mice or Cplx1+/− mice (Fig. 1B).

Cplx1−/− mice failed in the acquisition of the social transmission of food preference task

The social transmission of food preference task is a measure of cognitive function that exploits a natural tendency for mice to eat foods they know to be safe (because these foods have been eaten by a conspecific that has survived the experience). The social transmission of food preference task was used here because it is a ‘cognitive’ task (30), but does not rely on co-ordinated motor function for an outcome. Nevertheless, additional measures were taken to ensure that the motor impairments of Cplx1−/− mice did not confound this task. Accessibility of the flavoured foods for Cplx1−/− mice was increased by placing the food in low jars, so the impaired animals did not have to rear up in order to eat. We were satisfied that Cplx1−/− mice did not have a problem eating the food in this experiment, as the total amount of food eaten by Cplx1−/− mice was greater than that eaten by Cplx1+/+ mice.

After presentation of the cued food, Cplx1+/+ mice learnt the task and ate a significantly greater amount of the cued food than the non-cued food. In contrast, Cplx1−/− mice failed to learn the task and in fact ate significantly more of the non-cued food (Fig. 2A, genotype × food choice, F_{1,23} = 35.25, P < 0.001). The failure of Cplx1−/− mice in this task is consistent with lack of social learning. We added a further step to this task, in which the mice were presented with a new ‘safe’ food. Pilot studies using wild-type mice showed that they would either transfer their food preference to the novel cued food or eat similar amounts of both safe foods. Cplx1+/+ mice showed a trend for a preference to the new cued food, although this did not reach significance (P = 0.093). In contrast, Cplx1−/− mice ate significantly more of the previously-cued food than of the novel cued food (Fig. 2B, genotype × food choice, F_{1,23} = 29.71, P < 0.001). Note that in both parts of this test, Cplx1+/+ mice ate significantly more food in total than Cplx1+/+ mice. In the initial trial, Cplx1+/+ mice ate 0.32 ± 0.06 g of food in total compared with 0.50 ± 0.06 g eaten by Cplx1−/− mice (P = 0.65); in the transfer trial, Cplx1+/+ mice ate 0.37 ± 0.07 g of food compared with 0.71 ± 0.08 g of food eaten by Cplx1−/− mice (P < 0.05). The preference for the non-cued food shown by Cplx1−/− mice in the transfer trial is likely to reflect the lack of transfer of the food preference. Further, the lack of a preference for the new cued food in the second trial suggests a preference for the familiar food (rather than an aversion to the novel food). In the first trial, Cplx1−/− mice ate more of the non-cued food than the cued food, although they ate the same total amount of food as wild-type mice. Before this experiment, they had not been previously exposed to either food flavour. If Cplx1−/− mice had an aversion to novel food, they should have eaten less in the first trial, not more.

Although the social transmission of food preference task has been used by others to measure cognitive function (31–33), observation of the mice during this task suggested that the poor performance of Cplx1−/− mice in this task was not necessarily due to deficits in cognitive function. Thus,
we considered alternative explanations for failure in this task, namely that Cplx1−/− mice might have olfactory deficits or that Cplx1−/− mice might have deficits in social interactions that prevented them from adequately learning the task.

**Cplx1−/− mice have normal olfactory discrimination and olfactory memory**

To test whether Cplx1−/− mice had deficits in olfaction, we used a simple olfactory discrimination task. Normally, wild-type mice prefer to eat a familiar food (as opposed to a novel food). Here Cplx1−/− mice were trained to eat mint-flavoured food for 4 days. On the fifth day, they were given a choice between mint-flavoured (familiar) food and oregano-flavoured (novel) food. Cplx1−/− mice demonstrated that they were able to discriminate between two different olfactory cues since they ate significantly more of the familiar food than the novel food (Fig. 2C, \( P < 0.001 \)). The ability of Cplx1−/− mice to discriminate between the two different foods suggested that impairments in olfaction in Cplx1−/− mice were not responsible for the failure of Cplx1−/− mice in the social transmission of food preference task. The fact that Cplx1−/− mice ate significantly more of the cued food also suggested that Cplx1−/− mice could remember an olfactory cue for at least 24 h (the time since they had last seen the familiar food). Since in the second part of the transmission of food preference task, Cplx1−/− mice ate more of the previously cued food (with which they had been presented 4 days previously) it seemed likely that olfactory memory was normal in these mice.

During the social interaction phase of the social transmission of food preference experiment, we observed that there were considerable differences in the manner of interactions between observer and demonstrator mice of different genotypes. When a Cplx1+/+ observer mouse was introduced to a Cplx1+/+ demonstrator mouse, there was an immediate and obvious interaction between the two animals. The observer mouse would often hold the muzzle of the demonstrator mouse and repeatedly sniff its mouth and face. The observer mouse would also sniff the flanks and underbelly of the demonstrator mouse. These types of interactions were never observed in Cplx1−/− mice. In fact, Cplx1−/− mice appeared to be unaware of the presence of the demonstrator mouse. We do not think that this lack of interaction was due to the ataxia or other locomotor deficit, because despite their ataxia, the Cplx1−/− mice are physically capable of the motor function necessary for such interactions. (See Video, where the Cplx1−/− mouse is exploring the open field). Rather, we suggest that their failure in the social transmission of food preference task may be due to a deficit in social interaction. We therefore decided to investigate the social behaviour of Cplx1−/− mice directly.

**Cplx1−/− mice have deficits in preference for social novelty**

We tested Cplx1−/− mice in a three-compartment box (Fig. 3) that was designed to test two different facets of social behaviour; sociability and preference for social novelty (33).
one of three chambers. In the first trial, chamber 1 contained a ‘stranger’ mouse under a wire cup, and chambers 2 and 3 were empty. In the first trial, Cplx1<sup>+/+</sup> mice spent a significantly greater proportion of time in the chamber containing the stranger mouse than in either of the empty chambers (Fig. 4A, time spent in chamber,  F<sub>1,30</sub> = 14.23,  P < 0.001). This is typical of wild-type mouse behaviour. Cplx1<sup>2/2</sup> mice behaved similarly, spending significantly more time in the chamber containing the stranger mouse (Fig. 4A, time spent in chamber,  F<sub>1,30</sub> = 14.228,  P < 0.001). However, in this trial, Cplx1<sup>+/+</sup> mice made a significantly greater number of approaches than Cplx1<sup>2/2</sup> mice to the wire cup containing the stranger mouse. Cplx1<sup>+/+</sup> mice made 13.93 ± 0.95 approaches compared with 9.0 ± 0.68 approaches made by Cplx1<sup>2/2</sup> mice,  P < 0.001.

The second part of the test was designed to look at the preference for social novelty of the test mouse. The test mouse was removed to another cage. The mouse under the cup in compartment 1 was left in place, and a second, ‘novel’ stranger mouse was placed under a wire cup in the compartment 3. The test mouse was then returned to the central chamber and allowed to explore freely the whole box. As expected (33), Cplx1<sup>+/+</sup> mice spent significantly more time in the chamber containing the novel stranger mouse than the ‘familiar’ stranger mouse (Fig. 4B, genotype × time spent in chamber,  F<sub>1,30</sub> = 6.038,  P < 0.05). Cplx1<sup>+/+</sup> mice also made significantly more approaches to the novel stranger mouse (Fig. 4C, stranger,  F<sub>1,30</sub> = 6.94,  P < 0.01). However, Cplx1<sup>−/−</sup> mice spent a similar amount of time in both chambers (Fig. 4B, time spent in chamber,  F<sub>1,30</sub> = 2.58,  P = 0.55) and made a similar number of approaches to both the familiar stranger and novel stranger mice (Fig. 4C, stranger,  F<sub>1,30</sub> = 6.94,  P = 0.83) indicating a lack of preference for social novelty. As in the first part of the test, the total number of entries made into either chamber in the second part of the task was significantly lower (P < 0.001) for Cplx1<sup>−/−</sup> mice (7.9 ± 1.0 entries) than for Cplx1<sup>+/+</sup> mice (13.4 ± 0.81 entries).

In addition to the data showing that Cplx1<sup>−/−</sup> mice have deficits in preference for social novelty, the observed behaviour of Cplx1<sup>−/−</sup> mice was very different to that of Cplx1<sup>+/+</sup> mice throughout the experiment. Although

Figure 3. The sociability and social novelty testing apparatus. The mouse to be tested (test mouse) was first habituated to the central compartment (2). Then a ‘stranger’ mouse was placed in compartment 1, underneath the wire cup. For the second part of the experiment, a second ‘stranger’ mouse was placed underneath the wire cup in compartment 3.

Figure 4. Cplx1<sup>−/−</sup> mice have decreased preference for social novelty. Cplx1<sup>+/+</sup> mice and Cplx1<sup>−/−</sup> mice spent a significantly greater amount of time in the chamber containing the stranger than in the empty chamber (A). When a second stranger mouse was introduced to the apparatus Cplx1<sup>+/+</sup> mice spent significantly longer in the chamber containing the novel stranger compared to the chamber containing the familiar stranger, whereas Cplx1<sup>−/−</sup> mice spent a similar amount of time in each chamber (B). Cplx1<sup>−/−</sup> mice made fewer approaches to the novel stranger than Cplx1<sup>+/+</sup> mice (C). Bars represent mean ± SEM. Asterisks indicate significant differences (*P < 0.05, **P < 0.01).
Cplx1<sup>−/−</sup> mice spent more time in the chamber with the stranger mouse than in the empty chamber, their behaviour was not the same as the wild-type mice. Whereas Cplx1<sup>+/+</sup> mice spent most of their time on or near the wire cups containing the stranger mice, Cplx1<sup>−/−</sup> mice would often sit in the corner of the chamber that was farthest away from the wire cup containing the stranger mouse. This difference is reflected in the few approaches Cplx1<sup>−/−</sup> mice made towards the stranger mouse (Fig. 4C).

The poor performance of Cplx1<sup>−/−</sup> mice in the sociability task is not due to their motor impairments

It was possible that the deficits of Cplx1<sup>−/−</sup> mice seen in the sociability task were due to their locomotor impairments, which prevented them from effectively sampling the areas around the cups. To examine this directly, we tested Cplx1 mice in the apparatus used for the sociability task using food cues under the wire cup rather than mice. In the habituation phase (with two empty cups), there were no differences in the amount of time spent in either chamber between Cplx1<sup>+/+</sup> mice and Cplx1<sup>−/−</sup> mice (Fig. 5A). However, Cplx1<sup>+/+</sup> mice made significantly more approaches to, and spent more time interacting with the wire cups than Cplx1<sup>−/−</sup> mice (Fig. 5C, genotype, F<sub>1,14</sub> = 8.48, P < 0.01; Fig. 5E, genotype, F<sub>1,14</sub> = 10.79, P < 0.01). This reflects the generally lower levels of exploration seen in Cplx1<sup>−/−</sup> mice in the sociability task and reported previously (11).

In the next phase of the experiment, food was placed under one of the empty cups and the test mouse was allowed to explore the arena freely. Both Cplx1<sup>+/+</sup> mice and Cplx1<sup>−/−</sup> mice spent significantly more time in the chamber containing the food rather than in the chamber containing the empty cup (Fig. 5B, chamber, F<sub>1,14</sub> = 264.65, P < 0.001). Similarly, both Cplx1<sup>+/+</sup> mice and Cplx1<sup>−/−</sup> mice made a greater number of approaches to the wire cup containing the food than the empty wire cup (Fig. 5D, cup, F<sub>1,14</sub> = 100.97, P < 0.001) and spent significantly more time interacting with the cup containing the food than with the empty wire cup (Fig. 5F, cup, F<sub>1,14</sub> = 51.34, P < 0.001). This indicates that Cplx1<sup>−/−</sup> mice are able to discriminate between two choices (food or no food) and can respond to that choice accordingly despite their motor impairments. These data support the idea that the results of the sociability task were due to a deficit in social behaviour rather than to the inability of Cplx1<sup>−/−</sup> mice to perform the task adequately.

Cplx1<sup>−/−</sup> mice show reduced responsiveness in the resident intruder task

We used a resident intruder paradigm to investigate the innate territorial behaviours normally seen in male rodents. Cplx1<sup>−/−</sup> mice showed marked behavioural abnormalities in the resident–intruder paradigm (Fig. 6). Cplx1<sup>−/−</sup> resident mice displayed significantly fewer aggressive episodes towards both Cplx1<sup>+/+</sup> and Cplx1<sup>−/−</sup> intruders in the first part of the test (when the barrier was present) than Cplx1<sup>+/+</sup> mice (Fig. 6A, genotype, F<sub>1,29</sub> = 34.63, P < 0.001). Cplx1<sup>−/−</sup> resident mice also showed significantly fewer aggressive episodes towards Cplx1<sup>−/−</sup> intruder mice than Cplx1<sup>+/+</sup> mice in the second part of the test when the barrier was removed. Interestingly, Cplx1<sup>+/+</sup> resident mice showed significantly more aggression towards Cplx1<sup>−/−</sup> intruder mice than towards Cplx1<sup>+/+</sup> intruder mice when the barrier was removed (Fig. 6B, intruder, F<sub>1,29</sub> = 10.61, P < 0.001). This suggests that Cplx1<sup>+/+</sup> mice perceive Cplx1<sup>−/−</sup> mice as being more of a threat than Cplx1<sup>+/+</sup> mice, although the reasons for this are not clear.

Reductions in sniffing behaviours were seen in Cplx1<sup>−/−</sup>-resident mice compared with Cplx1<sup>+/+</sup> resident mice both in the first part of the test when a Cplx1<sup>−/−</sup> intruder mouse was present (Fig. 6C, genotype, F<sub>1,29</sub> = 19.41, P < 0.01) and in the second part of the test when that barrier was removed and the resident mouse and intruder mouse could interact directly (Fig. 6D, genotype, F<sub>1,29</sub> = 70.67, P < 0.001). This further supports the idea that Cplx1<sup>−/−</sup> mice do not readily initiate social contact and have severe impairments in social functioning.

Compared with Cplx1<sup>+/+</sup> mice, Cplx1<sup>−/−</sup> mice displayed reduced exploratory behaviours in the first part of the test irrespective of the genotype of the intruder mouse present on the other side of the barrier (Fig. 6E, genotype, F<sub>1,29</sub> = 88.49, P < 0.001). Cplx1<sup>−/−</sup> mice also displayed reduced social exploratory behaviours compared with Cplx1<sup>+/+</sup> resident mice when the barrier was removed and they were faced with a Cplx1<sup>−/−</sup> intruder (Fig. 6F, genotype, F<sub>1,29</sub> = 14.9, P < 0.001). Interestingly, Cplx1<sup>+/+</sup> mice showed significant reductions in social exploratory behaviours towards Cplx1<sup>−/−</sup> intruders compared with Cplx1<sup>+/+</sup> intruders in both the first (Fig. 6E, genotype × intruder, F<sub>1,19</sub> = 5.943, P < 0.05) and the second part of the task (Fig. 6F, genotype × intruder, F<sub>1,29</sub> = 18.39, P < 0.001). However, this reduction in social exploratory behaviours of Cplx1<sup>+/+</sup> mice is likely to be due, at least in part, to a concurrent increase in other behaviours engaged in by Cplx1<sup>−/−</sup> mice when there was a Cplx1<sup>−/−</sup> intruder, such as sniffing and aggressive behaviours. It may also be due to a lack of responsiveness on the part of the Cplx1<sup>−/−</sup> mice.

Cplx1<sup>−/−</sup> mice do not show increased anxiety in the elevated O-maze

It is possible that the deficits in social behaviour described earlier were due to increased levels of anxiety of ‘fearfulness’ in Cplx1<sup>−/−</sup> mice. The abnormal habitation behaviours already described in these mice would also indicate this possibility, even though traditional markers of anxiety in the open field paradigm were not elevated in Cplx1<sup>−/−</sup> mice (11). We therefore decided to test anxiety in Cplx1<sup>−/−</sup> mice directly in the elevated O-maze.

Cplx1<sup>−/−</sup> mice entered the open arm of the maze significantly quicker than Cplx1<sup>+/+</sup> mice (Fig. 7A, P < 0.01). Cplx1<sup>−/−</sup> mice also spent significantly more time in the open arms of the maze than Cplx1<sup>+/+</sup> mice (Fig. 7B, P < 0.01). We qualified stretched attend postures that are a marker of risk assessment in maze tasks and decrease in the elevated O-maze in response to anxiolytic drugs (34). Cplx1<sup>−/−</sup> mice showed significantly fewer stretched attend
postures while moving from the closed arm towards the open arm than Cplxl+/mice (Fig. 7C, P < 0.001). Together these data indicate that Cplxl−/−mice do not have an anxiety-like phenotype, or that if they do, this is not manifest as the beha-

vioural responses that typically reflect anxiety in wild-type mice.

**Cplxl−/− mice show impulsivity in the visual cliff avoidance task**

The behaviour of Cplxl−/−mice was examined using the visual cliff avoidance task. Mice were placed on a small platform and allowed to explore the test arena. There were no
differences between the two genotypes in the latency to descend the platform (Fig. 8A). However, significantly fewer Cplx1\(^{−/+}\) mice made their first foot fall on the bench side of the platform than Cplx1\(^{++}\) mice (Fig. 8B, \(X^2 = 14.81, P < 0.001\)) and instead stepped onto the cliff side of the platform. Typically, wild-type mice will be wary of the cliff and remain on the bench side of the testing arena, although after exploration of the arena, normal mice will learn that the ‘cliff’ side of the testing arena is safe to walk on and eventually they cross over the divide to explore the whole testing arena. Cplx1\(^{−/−}\) mice crossed over to the cliff side of the box significantly quicker than Cplx1\(^{++}\) mice (Fig. 8C, \(F_{2,47} = 10.35, P < 0.05\)). Before Cplx1\(^{++}\) mice crossed the cliff, they tentatively approached the divide and ‘tested’ the cliff by carefully placing a paw on the cliff side. However, Cplx1\(^{−/−}\) mice walked straight over to the cliff side without first investigating the cliff. This would suggest that Cplx1\(^{−/−}\) mice are...
If anything, they are impulsive, taking little time to fully explore a situation before acting. *Cplx1*-*/-* mice spent more time in total on the cliff side of the testing arena than *Cplx1*+/*+* mice (Fig. 8D, $F_{2,47} = 25.72$, $P < 0.001$).

The deficits in social interactions we described earlier could be explained by a neophobia in *Cplx1*-*/-* mice. We decided to test this directly with a novel object recognition paradigm. *Cplx1*-*/-* mice show no evidence of neophobia.
In the first trial, there was a significant effect of genotype on the amount of time spent exploring the two identical novel objects (Fig. 9A, genotype, \( F_{1,34} = 12.11, P < 0.001 \)), with \textit{Cplx1}^{+/+} mice spending more time exploring object 2 than \textit{Cplx1}^{−/−} mice (\( P < 0.05 \)). \textit{Cplx1}^{−/−} mice spent more time investigating object 1 than object 2 (\( P < 0.05 \)). There was no difference in the amount of time spent exploring either object for \textit{Cplx1}^{+/+} mice (\( P = 0.233 \)). There was a significant difference between genotypes in the total amount of time spent exploring the objects (\( F_{1,34} = 34.18, P < 0.001 \)). \textit{Cplx1}^{−/−} mice spent less time investigating object 1 than object 2 (\( P < 0.05 \)). There was no difference in the amount of time spent exploring either object for \textit{Cplx1}^{+/+} mice (\( P = 0.233 \)). There was a significant difference between genotypes in the total amount of time spent exploring the objects (\( P < 0.01 \)), and \textit{Cplx1}^{+/+} mice made fewer approaches to the objects than \textit{Cplx1}^{−/−} mice (Fig. 9B, genotype, \( F_{1,34} = 20.67, P < 0.001 \)). This is consistent with the exploratory deficits we have described previously. When object 2 was removed and replaced with a novel object, both \textit{Cplx1}^{+/+} mice and \textit{Cplx1}^{−/−} mice spent significantly more time exploring the novel object than the familiar object (Fig. 9C, object, \( F_{1,54} = 20.67, P < 0.001 \)). Also, there was no difference between the genotypes in the total amount of time spent exploring the objects (\( P = 0.946 \)). \textit{Cplx1}^{+/+} mice made significantly more approaches to the novel object than object 1 (Fig. 9D, genotype, \( F_{1,34} = 18.2, P < 0.05 \)). These data show that although \textit{Cplx1}^{−/−} mice show reduced exploration of novel stimuli, they are able to recognize a novel object. These findings are consistent with our earlier work (11) and suggest that \textit{Cplx1}^{−/−} mice are not neophobic.

**DISCUSSION**

\textit{Cplx1}^{−/−} mice have a severe ataxia coupled with dystonia and tremor in the absence of neurodegeneration (3,11). In addition to their motor impairments, \textit{Cplx1}^{−/−} mice also have profound exploratory and habituation deficits (11). Here we investigated the cognitive and social behavioural phenotype of \textit{Cplx1}^{−/−} mice. We found that \textit{Cplx1}^{−/−} mice have major impairments in natural social behaviours. However, although cognitive testing was severely limited owing to the motor impairments of \textit{Cplx1}^{−/−} mice, in a paradigm where motor disability did not interfere with cognitive testing, their cognitive abilities appeared to be intact.

Because adult \textit{Cplx1}^{−/−} mice cannot swim, we thought that cognitive testing in a swimming-dependent task would be...
impossible. However, by teaching juvenile Cplx1\(^{-/-}\) mice to swim, we were able to assess their cognitive function in a simple two-choice discrimination task, the two-choice swim tank. We found that although their poor swimming style increased the time taken to perform the task, Cplx1\(^{-/-}\) mice had neither acquisition nor reversal learning deficits. In fact, their performance in this task was as good as that seen in Cplx1\(^{+/+}\) and Cplx1\(^{+/+}\) mice. This is interesting because Cplx2\(^{-/-}\) mice (that can swim perfectly well) have a major deficit in the reversal phase of this task (10).

The social transmission of food preference task has been widely used as a test for learning and memory (32,35,36). We used this task to investigate further the cognitive abilities of Cplx1\(^{-/-}\) mice. Cplx1\(^{-/-}\) mice failed to show a preference for the food cued by the demonstrator mouse, suggesting that the mice had cognitive deficits. Given that performance in the two-choice swim tank task was normal, we wondered whether there was an alternative explanation for their poor performance in the social transmission of food preference task. There were three obvious possibilities. First, the ataxia of Cplx1\(^{-/-}\) mice could have prevented them from performing this task; secondly, the mice might have olfactory deficits; thirdly, the mice may have abnormal social behaviour. We ruled out the possibility that the poor performance was due to ataxia. Although the motor impairment of Cplx1\(^{-/-}\) mice was severe, they were able to move around the testing environment. Further, the apparatus used for the task was modified so that Cplx1\(^{-/-}\) mice could perform the mechanics of the task. We also ruled out olfactory impairment as an explanation for the failure of Cplx1\(^{-/-}\) mice in this task. Cplx1\(^{-/-}\) mice can distinguish between different olfactory cues, and olfactory memory appears to be intact. However, we could not rule out abnormal social behaviours. First, home cage observations showed that Cplx1\(^{-/-}\) mice exhibited abnormal social interactions. Secondly, direct measurement of social behaviours confirmed that the failure in the social transmission of food preference task is more likely to due to social rather than cognitive impairments. Although we cannot rule out the possibility that Cplx1\(^{-/-}\) mice have cognitive deficits that may contribute to their poor performance, deficits in social behaviours are the most plausible explanation for the failure of Cplx1\(^{-/-}\) mice in the social transmission of food preference task.

It remains possible that the failure of Cplx1\(^{-/-}\) mice to learn the social transmission of food preference task is due to a cognitive impairment. This task has been recognized as a non-spatial learning paradigm that requires an intact hippocampus and related structures (30,35,37). In contrast, learning in the two-choice swim tank (that was normal in the Cplx1\(^{-/-}\) mice) is thought to be predominantly dependent upon striatal function (38–40). Unfortunately, we have not been able to test hippocampal-dependent learning directly. This is because we have not yet found a task that measures hippocampal function that Cplx1\(^{-/-}\) mice are capable of performing. Hippocampal-based learning is usually measured using spatial learning paradigms such as the Morris water maze (41). However, this task requires good swimming ability. Although we have demonstrated that juvenile Cplx1\(^{-/-}\) mice are able to learn to swim well enough to perform in the two-choice swim tank, the swimming requirements of the water maze are much greater, and testing of Cplx1\(^{-/-}\) mice in the Morris water maze is not possible. Spatial memory in Cplx1\(^{-/-}\) mice could be measured using a ‘dry’ learning paradigm such as Barne’s maze or radial arm maze. However, the exploratory deficits seen in Cplx1\(^{-/-}\) mice (11, this study) prevent Cplx1\(^{-/-}\) mice from performing these tasks adequately. Further, pilot experiments from our laboratory show that Cplx1\(^{-/-}\) mice fail to perform well in the radial arm maze because they are impulsive and this adds a confounds makes interpretation of the data difficult. Also, Cplx1\(^{-/-}\) mice do not initiate nosepoke activities (unpublished data), so testing in a paradigm such as Barne’s maze (42) is not possible. When a task becomes available that tests spatial memory but does not rely on either coordinated motor function or exploratory strategies, it would clearly be interesting to investigate more closely the hippocampal-dependent cognitive behaviour in Cplx1\(^{-/-}\) mice.

Although there are alternative explanations for the failure of Cplx1\(^{-/-}\) mice in the tasks measuring social behaviour, it is likely that deficits in social behaviours cause the failure of Cplx1\(^{-/-}\) mice in the social transmission of food preference task because when we tested social interactions directly, the Cplx1\(^{-/-}\) mice showed marked deficits. Social interactions form an intrinsic element of rodent behaviour. They are important for both maternal and mating behaviours (43). The social behaviour of male mice is largely aggressive and territorial in nature, as they rely on social dominance to attract females with which to mate. These ‘normal’ social behaviours were not seen in Cplx1\(^{-/-}\) mice. Cplx1\(^{-/-}\) male mice showed little aggression or interest in other mice in the resident–intruder task and they failed to show normal social investigations in the sociability and social preference task.

It is possible that the motor impairments of Cplx1\(^{-/-}\) mice prevent them from performing the tasks as required. However, Cplx1\(^{-/-}\) mice are physically capable of all the actions required to perform in the social transmission of food preference task. Although impaired, Cplx1\(^{-/-}\) mice can maintain the balance required to interact with a conspecific, and they remain stable when they are stationary. They have the co-ordination necessary for exploring when they want to (Video). Thus they should be physically capable of interacting with the observer mouse and detecting the cued food on their breath. Similarly, Cplx1\(^{-/-}\) mice were physically capable of performing the sociability and resident–intruder tasks. This was demonstrated clearly when food was used as a cue instead of another mouse. The increased exploration of the chamber and the wire cup containing the food compared with the empty chamber and wire cup clearly demonstrates that Cplx1\(^{-/-}\) mice can discriminate between two choices offered and that they could perform the task despite their motor impairments. In the sociability task, although Cplx1\(^{-/-}\) mice moved between the chambers less frequently than Cplx1\(^{+/+}\) mice, they were not stationary and did move between the chambers. However, unlike Cplx1\(^{+/+}\) mice, which actively investigated the stranger mice in the wire cups, Cplx1\(^{-/-}\) mice briefly investigated the stranger mice but spent the majority of the time exploring other areas of the chamber. This would indicate that the deficit represents impairment in social exploration rather than locomotion.
An alternative possible explanation for the failure of Cplx1\(^{-/-}\) mice in the sociability task that has not been elucidated is that they have olfactory impairments that prevent them from discriminating between different mice. Although Cplx1\(^{-/-}\) can discriminate between two different foods, we cannot rule out the possibility that it may not be able to discriminate between two different mice. Cplx1 is highly expressed in the olfactory bulbs (www.stjudesbegm.org/web/view/mediumImage/viewMediumImage.php?slideId=11129) (9), which are known to be important for pheromone detection in rodents (44). We are planning to test this in the future.

Another explanation for our findings is that the mice are neophobic, and this impairs their performance in the social transmission of food preference task. Previous work supports the idea that Cplx1\(^{-/-}\) mice may have an anxiety-related phenotype that could include neophobia (11). However, differentiating between anxiety, fearfulness and neophobia in mice is difficult (45). Three different free exploration paradigms were used in an attempt to examine this question. Direct testing of anxiety in Cplx1\(^{-/-}\) mice in the elevated O-maze showed that Cplx1\(^{-/-}\) mice appeared much less ‘anxious’ than Cplx1\(^{+/+}\) mice. Cplx1\(^{-/-}\) mice explored the open areas of the maze and entered there readily, making few stretched attend postures. Similarly, the readiness with which Cplx1\(^{-/-}\) mice crossed onto the cliff in the visual cliff avoidance task indicates that these mice are also not fearful of novel environments (in fact, quite the opposite). It is possible that the response of Cplx1\(^{-/-}\) mice in the visual cliff discrimination task is due to poor eyesight in Cplx1\(^{-/-}\) mice. However, there is no evidence for this, as Cplx1\(^{-/-}\) mice can recognize and distinguish between two objects as seen in the novel object recognition task. Although we cannot rule out a role for Cplx1 in visual processing, Cplx1 is not expressed in the retina (46) and so a lack of Cplx1 should have no effect on retinal processing. We directly tested neophobia in Cplx1\(^{-/-}\) mice using the novel object recognition paradigm. We found that (as seen in Cplx1\(^{+/+}\) mice) Cplx1\(^{-/-}\) mice spent significantly more time exploring the novel object. This indicates that they are capable of recognizing a novel object and also that they do not have an aversion to novel stimuli. Further evidence against Cplx1\(^{-/-}\) mice being neophobic comes from the social transmission of food preference task, sociability or resident–intruder tasks. If they were neophobic, we would expect them to avoid eating novel foods in the social transmission of food preference task and to avoid contact with stranger mice in the sociability task. However, Cplx1\(^{-/-}\) mice ate more of the two novel foods in the first part of the social transmission of food preference task compared with Cplx1\(^{+/+}\) mice. Furthermore, although Cplx1\(^{-/-}\) mice showed reduced contact in the sociability and resident–intruder paradigms, they did not show signs of fear. No tail rattling, retreat or escape responses were observed from Cplx1\(^{-/-}\) mice in these tasks. Together, the data we obtained in the elevated O-maze, the visual cliff avoidance task and the novel object recognition task suggest that the data from the social behaviour tests are best explained by deficits in social behaviours.

Our findings of abnormal social behaviour in Cplx1\(^{-/-}\) mice are interesting because the expression of Cplx1 is dysregulated in three important neurological diseases [schizophrenia (19–22), major depressive disorder (23) and bipolar disorder (24,25)], in which impairments in ‘social cognition’ are key components of the disease. Social cognition is the ability to process social cues and information and then to apply these appropriately according to social situations. To do this successfully, an individual must be able to understand emotion in others, correctly perceive and interpret the emotional displays of others and recognize social cues and social roles (47). All three of these elements of social cognition are disrupted in schizophrenia (48–50) and it has been proposed that disruptions in prefrontal-thalamic and cerebellar circuitry may account for the many and varied symptoms of schizophrenia (51). Notably, Cplx1 is highly expressed in both the thalamus and cerebellum (8), and both Cplx1 and Cplx2 are dysregulated in schizophrenia. Further, people who suffer from depression have blunted social interactions and exhibit marked social withdrawal (52), and patients with bipolar disorder also show poor social functioning (53,54). Again, both isoforms of complexin are dysregulated in major depression and bipolar disorder. The social abnormalities seen in Cplx1\(^{-/-}\) mice (lack of interest in other mice, social apathy) are also very reminiscent of the social deficits associated with autistic spectrum disorders (ASD). It is not known whether complexin expression is abnormal in ASD; to our knowledge, this has not been examined.

There is as yet no direct genetic link between any of the diseases in which social function is impaired, although these diseases have clinically overlapping signs and symptoms and there is considerable evidence showing that schizophrenia and bipolar disorder are part of a continuous spectrum of disease, with some genes contributing to both disorders (55). Further, there is currently no evidence for a causal involvement of Cplx1 in any of these diseases, although it has been suggested that polymorphisms in CPLX2 may confer susceptibility to schizophrenia in a Korean population (56). Polymorphisms in the Cplx1 gene have been examined, but are not linked to schizophrenia (57). To our knowledge, the complexin genes have not been examined as risk factors for bipolar disorder, AD or ASD.

Interestingly, there are four mouse models that we have identified in the literature in which social behavioural abnormalities similar to those seen in Cplx1\(^{-/-}\) mice have been described. These are the fragile X mental retardation gene (Frm1) knockout mice, ENGRAILED 2 knockout (En2\(^{-/-}\)) mice, heterozygous neuregulin-1 knockout (Nrg-1 KO) mice and haploinsufficient (+/rl) reeler mice (HRM). The Frm1 knockout mouse is a model of Fragile-X syndrome and ASD (58,59) that displays deficits (decreases) in aggressive behaviours (60) and marked impairments in social interactions (61). ENGRAILED 2 is a susceptibility gene for ASD and En2\(^{-/-}\) mice show impairments in spatial learning as well as social behaviours such as reduced aggression in a resident intruder paradigm and reduced play type behaviours in juvenile mice (62). Neuregulin-1 is a susceptibility gene for schizophrenia (63) and heterozygous Nrg-1 KO mice have exploratory deficits (64) and abnormal responses to social novelty (65) similar to those seen in Cplx1\(^{-/-}\) mice. Reelin is altered in the cerebellum of schizophrenic populations (66), bipolar disorder (67,68) and in autism (69). While HRM mice do not show social deficits, their motor abnormalities are very
similar to those seen in Cplx1<sup>−/−</sup> mice. It would be interesting to see if either of the complexins was dysregulated in any of these mice lines. The marked phenotypic similarities between Cplx1<sup>−/−</sup> mice and the mouse models described above suggest that despite the disparity between the genetic causes of these disorders, there may be a common downstream mediator of the functional abnormalities seen in these mouse models. The processes involved in the regulation of social behaviours and social cognition are complex (47,70). However, it is clear that intact neuronal circuitry is crucial for the proper identification, processing and response to socially relevant stimuli. A lack of Cplx1 may lead to altered synaptic functioning in the neuronal circuits involved in behaviours mediated by the thalamus, cortex and cerebellum. This is likely to contribute to the social deficits seen in the diseases where complexin expression is altered. We have suggested previously that Cplx2 may play a role in ASD (10) since Cplx2<sup>−/−</sup> mice have both subtle motor deficits and cognitive abnormalities. However, given the data we present here, it seems likely that abnormal expression of both complexin isoforms would contribute to the higher cortical dysfunction in all of the diseases in which complexins are dysregulated, particularly, since complexins are up- or down-regulated to varying degrees depending on the disease. In summary, the impairments in social behaviour of Cplx1<sup>−/−</sup> mice show a striking parallel to the abnormal social behaviours in humans with neurological diseases where complexin expression is altered, such as schizophrenia, major depressive illness, bipolar disorder and AD. These data support our previous work (10,11) suggesting that complexins play a critical role in the ‘higher’ neurological functions that allow an animal to express a full repertoire of adult behaviours.

**METHODS**

**Animals**

Cplx1 mice were created by homologous recombination in embryonic stem cells (3) and bred on a mixed genetic background (129Ola/C57Bl6). The mice used in this study (a gift of Drs N. Brose and K. Reim) were backcrossed on to the C57Bl6 inbred strain for three generations in the Department of Pharmacology, University of Cambridge. Cplx1 mice have now been backcrossed on to the C57Bl6 inbred background for eight generations and no change in the overt phenotype has been observed. Wild-type (Cplx1<sup>+/+</sup>) and homozygote knockout (Cplx1<sup>−/−</sup>) mice were generated by breeding from heterozygote (Cplx1<sup>+/−</sup>) pairs of mice. All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

All mice were housed in hard-bottomed polypropylene cages in groups of 10. The housing facility was maintained at 21–23°C, 55 ± 10% humidity on a 12 h light dark cycle. All mice were tested during the light phase. A total of 103 mice were used in this study (48 Cplx1<sup>+/+</sup> mice (32 male and 16 female), 12 Cplx1<sup>−/−</sup> mice (six male and six female) and 43 Cplx1<sup>+/−</sup> mice (32 male and 11 female)). All Cplx1 mice were housed according to sex and genotype except the Cplx1<sup>−/−</sup> mice used for the social transmission of food preference task that were housed with two Cplx1<sup>+/+</sup> female mice. All mice were provided with 8/10 grade corncob bedding, shredded paper nesting material and limited environmental enrichment in the form of a red polycarbonate igloo house with Fast-trac running wheel and a wooden tunnel (all from Datesand, Manchester, UK). All animals were given ad libitum access to standard dry laboratory food and water. As Cplx1<sup>−/−</sup> mice have difficulty in rearing, all Cplx1 mice were provided with lowered waterspouts and twice daily supplementary feeding. Supplementary feeding consisted of softened chow pellets (created by soaking 100 g dry chow in 230 ml water) placed on the floor of the home cage twice daily (71).

**Genotyping.** DNA was extracted from tail biopsies as described in reference (11). The presence of Cplx1<sup>+/+</sup> or Cplx1<sup>−/−</sup> genes was detected by polymerase chain reaction (PCR). Primers used for Cplx1<sup>+/+</sup> gene were: forward 5′-AGTACTTTTGAATCCCTGTTGA-3′ and reverse 5′-TAGCTATCCCTTCTTGTCCTTGT-3′. Primers used for the Cplx1<sup>−/−</sup> gene were: forward 5′-CGCCGCGGGATTGTGACCTC-3′ and reverse 5′-CTGCGTTGTCCTCCTAAGTCCTGTG-3′. PCR was conducted as described in reference (72) using 0.3 ng DNA. The cycling conditions used were: 3′ at 94°C, 45 × (30′ at 94°C, 30′ at 53°C, 1′ at 72°C) and 7′ at 72°C. The PCR products obtained were then run on a 1.5% agarose gel for 1 h at 145v alongside φX174 DNA/Hae III marker (Promega, WI, USA) and visualized under ultra-violet light.

**Behavioural testing**

**Two choice swim tank.** At 4.3 weeks ± 5 days of age, naïve Cplx1<sup>+/+</sup> mice (n = six male, six female), Cplx1<sup>−/−</sup> mice (n = six male, six female) and Cplx1<sup>+/−</sup> mice (n = seven male, three female) were trained to swim. They were then tested in a simple light dark visual discrimination task as previously described (28). The two-choice swim tank is a water filled glass tank 100 cm × 30 cm × 16.5 cm. An area 20 cm long is marked in the centre of the tank and constitutes the start area. A platform was placed at one end of the tank and the tank was filled with water to a level 5 mm above the top of the escape platform. The position of the platform was randomly assigned so that there was no directional bias. The tank was enclosed in a white box (120 cm × 46 cm × 36 cm) to eliminate spatial cues. A 60 W desktop lamp was positioned at the same end of the tank as the platform for acquisition training. The light was visible through a 5 cm diameter hole in the end of the box. For acquisition training the light was positioned at the end of the platform with the tank so animals had to learn to swim towards the light. The platform was assigned to the dark end of the tank in reversal learning so that the mice had to swim away from the light to reach the platform. In both acquisition and reversal learning, animals were placed in the start area of the tank, facing the sidewall. For swimming training the mice were slowly lowered into the water over 4–5 s. This allowed them to keep their body as horizontal to the water as possible and helped minimize the panic response in the Cplx1<sup>−/−</sup> mice.
During the task, Cplx1\(^{-/-}\) mice were given assistance to reach the platform if necessary but this was kept to a minimum. If at the end of 60 s the mouse had not reached the platform, it was placed on the platform and left for 10 s. If a mouse swam to the platform but could not mount it, the mouse was assisted onto the platform. If a mouse had trouble swimming and got into difficulties then it was removed from the tank immediately and placed on the platform for 10 s. The swimming direction and the latency to reach the escape platform were recorded for each mouse. A correct choice was recorded if the animal swam directly towards the light and reached the platform. An incorrect choice was recorded if: (i) the mouse swam directly to the dark end of the tank; (ii) the mouse swam towards the light but then turned around and crossed back through the start area and (iii) the mouse failed to reach the platform within 60 s. Each animal received 10 trials a day in two blocks of five sessions each. There was an inter-trial interval of 5 min and an inter-session interval of 2 h to avoid fatigue in the animals. Testing continued for all mice until a criterion of 90% correct choices was achieved. All animals had a rest day between acquisition and reversal learning.

**Social transmission of food preference.** Cplx1\(^{+/+}\) mice (n = three male, seven female) and Cplx1\(^{-/-}\) mice (n = 10 male, five female) aged 38 ± 1 weeks were used in the social transmission of food preference task. For the test two Cplx1\(^{+/+}\) demonstrator mice were removed from each test, cage and individually housed overnight with water but without food (18 h). The demonstrator mice were then placed into clean cages containing flavoured ground food in small glass jars (3.9 cm diameter, 3.4 cm high) (Solmedia, UK). The food was flavoured with either cocoa (2%w/w), cinnamon (1%w/w) or cumin (0.25%w/w) (34). We used these flavours because in pilot studies using wild type mice, no bias was shown for any of these flavours (data not shown). The glass jars were set in shallow Petri dishes so that food scattered by the digging of the mice was retained. Demonstrator mice were left to eat the cued food (cumin) for 1 h. Dishes were weighed before and after to measure how much food was eaten. The demonstrator mouse was then placed in a clean experimental cage (with corncob bedding but no nesting material). One at a time ‘observer’ mice from the same home cage of the demonstrator mouse were placed in the cage containing the demonstrator mouse and left there for 5 min. The observer mouse was then removed. After an interval of 15 min the sequence of interaction was repeated, with each observer mouse being placed with the second demonstrator mouse from the home cage. All observer mice were then returned to the home cage and demonstrator mice were individually housed. Six hours after the social interaction sessions the observer mice were food-deprived for 18 h (overnight). The following morning, each mouse was placed individually in a clean cage (45 cm × 28 cm × 12 cm) containing two ‘dishes’ in either corner at the back of the cage; one with cumin-flavoured food (cued) and the other containing cocoa-flavoured food (non-cued). As Cplx1\(^{-/-}\) mice have motor difficulties that prevent them for rearing normally, the food was placed in ‘dishes’ (bakelite jar lids (4 cm diameter, 0.5 cm high). These allowed Cplx1\(^{-/-}\) mice easy access to the food. Mice were allowed to eat for 1 h. Dishes were weighed before and after to determine the amount of food eaten. Food preference was calculated as amount of cued food eaten/total food eaten × 100 (% total).

Four days after the initial exposure to a cued food the demonstrator mice were given a new cued food (cinnamon), and observer mice were exposed to the demonstrator mice using the procedure described above. However, this time the observer mouse was presented with a choice between cinnamon-flavoured (the new-cued) or cumin-flavoured (the previously-cued) food. As before, dishes were weighed before and after the observer mouse had eaten. Food preference was calculated as amount of cued food eaten/ total food eaten × 100 (% total).

**Olfactory discrimination.** Cplx1\(^{-/-}\) mice (n = 10 male, five female) aged 40 ± 1 weeks were tested. All mice were food deprived overnight (18 h). Individual mice were placed in clean cages with a glass jar set in a Petri dish placed at the centre back of the cage. Glass jars were filled with mint-flavoured food (0.5%w/w). Mice were allowed to eat ad libitum for 1 h. This procedure was repeated for four consecutive days. On the fifth day they were presented with a choice between mint (familiar food) and a novel food, oregano (0.7%w/w) flavoured. Dishes were weighed before and after the trial and preference for each food was calculated as amount of cued food eaten/total food eaten × 100 (% total).

**Sociability and social novelty.** Naive Cplx1\(^{+/+}\) mice (n = six male, eight female) and Cplx1\(^{-/-}\) mice (n = 10 male, eight female) mice aged 22 ± 5 weeks were tested. The tests for sociability and social novelty were conducted as described in reference (33). Briefly, the testing apparatus was a clear Perspex box (45 cm × 30 cm × 21.5 cm) divided into three equal chambers by two Perspex walls. The three compartments (1, 2 and 3, Fig. 3) were connected by two small round doorways (3.5 cm diameter). The first part of the test was designed to measure sociability by recording the amount of interest the test mouse showed towards a ‘stranger’ mouse. Initially the test mouse was placed in the central chamber (with the doorways blocked off) and allowed to habituate for 5 min. The test mouse was removed to another cage out of sight of the test apparatus while a ‘stranger’ mouse was placed under a wire cup (8 cm diameter, 9.5 cm tall) in one of the side chambers. The test mouse was then placed back in the central chamber and allowed to explore all three chambers for 10 min.

The second part of the trial was designed to test for preferences for social novelty. For this a second ‘stranger’ mouse was placed underneath a wire cup in the second side chamber. The test mouse was placed back into the central chamber and allowed to explore the whole box freely for a further 10 min. Wild-type mice typically recognize the presence of a new and unfamiliar mouse and initially spent a greater proportion of their time investigating the novel mouse rather than the mouse with which they are already familiar (33). The latency to leave the central chamber, the amount of time spent in each chamber and the numbers of approaches towards the wire cup containing the ‘stranger’ mouse were recorded in each trial. For all tests the stranger...
mice used were age-matched male Cplx1+/+ mice. The testing apparatus was cleaned with 1% acetic acid after each mouse was tested and the sawdust on the floor of the apparatus was replaced with clean sawdust after each trial.

**Locomotor testing.** Naïve Cplx1+/+ mice (n = six male, two female) and Cplx1−/− mice (n = four male, four female) aged 27 weeks ± 2 days were used to test the locomotor ability of Cplx1 mice in a dry maze task. Animals were food deprived overnight for 16 h but supplementary water was provided in Petri dishes on the floor of the cage to ensure good access to water for Cplx1−/− animals. Mice were initially habituated to the testing environment (the same as was used in the sociability test) for 10 min in groups of four. Mice were then individually habituated to the testing environment for two 5-min sessions. The two empty wire cups were placed in the two end chambers and the test mouse was placed in the central chamber and allowed to explore the entire apparatus. The following parameters were recorded: (i) the number of approaches to each cup (defined as the mouse touching the cup with its nose or the nose being within 1 cm of the cup); (ii) the amount of time spent interacting with each cup; (iii) the number of entries to each chamber and (iv) the time spent in each chamber. After the two habituation sessions mice were removed from the testing apparatus and 100 g of softened chow pellets were placed under one of the wire cups. The placement of the food under the wire cup was randomized across trials to prevent place bias. The test mouse was then returned to the central chamber and allowed to explore all three chambers for 5 min. The activity of the test mouse was recorded as before. The testing apparatus was cleaned with 1% acetic acid after each mouse was tested and the sawdust on the floor of the apparatus was replaced with clean sawdust after each trial.

**Resident intruder task.** Naïve Cplx1+/+ male mice (n = 20) and Cplx1−/− male mice (n = 11) aged 22 ± 4 weeks were used in the resident intruder paradigm as described in reference (73). Briefly ‘resident’ mice were placed in their home cage (45 cm × 28 cm × 12 cm) containing a Perspex barrier in the centre dividing the cage into two. The barrier measured 24 cm × 17.5 cm and had four rows of holes (8 mm diameter) to allow mice on opposite sides of the barrier to sniff at each other. The first row of holes (five in total, 4 cm apart) were 3 cm from the bottom of the barrier to the centre of the hole. The next row of holes (six in total, 3.4 cm apart) were 2 cm above the first row. The subsequent two rows of holes were copies of the first two rows of holes. With the barrier present, the ‘resident’ mouse was allowed to habituate to one side of the cage for 5 min. The ‘intruder’ mouse was then placed in the other side of the cage and the behaviour of the resident mouse towards the intruder was scored for 5 min. Behaviours were divided into four main categories; aggressive, defensive, exploratory and other. The barrier was then removed and the resident and intruder mice were allowed to interact for a further 5 min. Again, the behaviour of the resident mouse towards the intruder mouse was recorded.

**Elevated O-maze task.** The O-maze was used to measure anxiety in Cplx1−/− mice. Cplx1+/+ mice (n = six male, six female), Cplx1−/− mice (n = nine male, 10 female) and Cplx1+/+ mice (n = 10 male, five female) aged 27 ± 1.5 weeks were tested. The testing apparatus consisted of a circular (46 cm outer diameter, 5.5 cm wide), and elevated (40 cm above ground level) runway divided into four quadrants. Two quadrants (on opposite sides of the maze) were designated as ‘closed arms’ with 11 cm high walls on both sides and the two remaining quadrants were designated as ‘open arms’ with a 5 mm lip on each side of the runway. Mice were placed individually into one of the closed arms. Parameters measured were (i) latency to enter an open arm; (ii) time spent in the open arms and (iii) the number of stretched-attend postures made towards the open arm. A cushion of wood wool (approximately 10 cm deep) was placed underneath the maze in case a mouse fell off the open part of the maze. During testing, some Cplx1−/− mice leaned too far over the edge of the runway. This caused them to lose their balance and they were unable to pull the front of their bodies back onto the runway. Therefore, if Cplx1−/− mice leaned over the edge of the maze so that only their hindquarters were in contact with the runaway they were replaced onto the runway at that point.

The maze was cleaned thoroughly with 1% acetic acid between each trial.

**Visual cliff discrimination.** Cplx1+/+ mice (n = 10 males, eight females), Cplx1−/+ mice (n = six males, 12 females) and Cplx1−/− mice (n = eight male, six female), aged 25 ± 2 weeks were tested in the visual cliff avoidance task (10). The experiment was conducted in an open top Perspex box measuring 60 cm × 60 cm × 15 cm, which was placed on the edge of a laboratory bench so that half of the box was on the bench (bench side) and the other half was 90 cm above the floor (cliff side). A checkerboard pattern of the same area as the Perspex box was placed under the box and on the floor and on the cliff side to emphasize the cliff drop off. Two 60 w anglepoise lamps were positioned 50 cm below and 30 cm above the box to illuminate both the bench and cliff sides of the box, whilst the main laboratory light source was dimmed. A Perspex platform (10 cm × 7 cm × 2 cm) was placed in the centre of the box on the bench side along the edge of the cliff to allow the mouse to view its surroundings. Each mouse was placed on the platform and observed for 5 min starting from placement on the platform. Each mouse received a single trial. The parameters measured were: (i) the direction of first foot step (cliff or bench); (ii) the latency to descend platform (defined as three paws stepping onto the box); (iii) the latency to cross over to the cliff side (defined as three or more paws over the cliff); (iv) the time spent over the cliff in the first 2 min and (v) the total time spent over the cliff.

The box and platform were cleaned with 1% acetic acid solution after each trial.

**Novel object recognition.** For the novel object recognition test, adult Cplx1+/+ (n = 10 males, 10 females), Cplx1−/+ (n = 10 males, nine females) and Cplx1−/− (n = six males, 12 females) with a mean age of 16 ± 2 weeks were used. The experiment was conducted using the apparatus used for the
open field testing. On day one, each mouse was placed in the open field and allowed to habituate to the environment for a period of 5 min. On the second day, two identical objects (Perspex tubes, 9 cm high × 3 cm diameter, filled with 0.5% Cresyl violet solution) were placed on one diagonal 12 cm from the corners and secured to the floor with Velcro. The two objects were arbitrarily labelled as object 1 and object 2. Individual mice were placed in the arena with the objects and were allowed to explore freely for 5 min (74,75). After this time, they were returned to their home cage for 1 h. In the next trial, the object in position 2 was replaced with a novel object (a plastic figure of a parrot, 3 cm × 5 cm × 8 cm). Mice were placed in the arena again and allowed to explore the objects for another 5 min. In both trials the parameters measured were: (i) the number of approaches to each object and (ii) the time spent exploring each object. The walls and floor of the box and the objects were cleaned with 1% acetic acid between each trial.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) with two within subject factors, with or without repeated measures depending on the experiment. A Newman–Keuls or Duncan’s post hoc test was used for two within subject factors and a Bonferroni’s or post hoc analysis was implemented where repeated measures had been used. For data that was not normally distributed, the ANOVA used was a Kruskal–Wallace analysis. Statistical analyses were performed using GraphPad Prism (Version 4.0, San Diego, CA, USA) and Statistica (Version 6.0, Tulsa, OK, USA). A critical value for significance of $P < 0.05$ was used throughout this study.

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