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Clorgyline-mediated reversal of neurological deficits in a Complexin 2 knockout mouse

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Complexin 2 is a protein modulator of neurotransmitter release that is downregulated in humans suffering from depression, animal models of depression and neurological disorders such as Huntington's disease in which depression is a major symptom. Although complexin 2 knockout (*Cplx2*$^{-/-}$) mice are overtly normal, they show significant abnormalities in cognitive function and synaptic plasticity. Here we show that *Cplx2*$^{-/-}$ mice also have disturbances in emotional behaviours that include abnormal social interactions and depressive-like behaviour. Since neurotransmitter deficiencies are thought to underlie depression, we examined neurotransmitter levels in *Cplx2*$^{-/-}$ mice and found a significant decrease in levels of noradrenaline and the serotonin metabolite 5-hydroxyindoleacetic acid in the hippocampus. Chronic treatment with clorgyline, an irreversible inhibitor of monoamine oxidase A, restored hippocampal noradrenaline to normal levels (from 60 to 97% of vehicle-treated *Cplx2*$^{+/+}$ mice, $P < 0.001$), and reversed the behavioural deficits seen in *Cplx2*$^{-/-}$ mice. For example, clorgyline-treated *Cplx2*$^{-/-}$ mice spent significantly more time interacting with a novel visitor mouse compared with vehicle-treated *Cplx2*$^{-/-}$ mice in the social recognition test (34 compared with 13%, $P < 0.01$). We were also able to reverse the selective deficit seen in mossy fibre-long-term potentiation (MF-LTP) in *Cplx2*$^{-/-}$ mice using the noradrenergic agonist isoprenaline. Pre-treatment with isoprenaline *in vitro* increased MF-LTP by 125% ($P < 0.001$), thus restoring it to control levels. Our data strongly support the idea that complexin 2 is a key player in normal neurological function, and that downregulation of complexin 2 could lead to changes in neurotransmitter release sufficient to cause significant behavioural abnormalities such as depression.

INTRODUCTION

Complexins are small, cytosolic proteins that bind to the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex to regulate synaptic vesicle exocytosis (1,2). Complexin 1 and 2 are the two major isoforms in the brain (1). From functional analyses using complexin knockout mice, it was concluded that complexins operate at a post-priming step in synaptic vesicle excocytosis by stabilizing SNARE complexes in a highly fusogenic 'super-primed' state (3,4). In contrast, studies on neuromuscular junctions of *Drosophila* complexin null mutants (5) and in other *in vitro* systems (6–9) revealed that complexin acts as a clamp that arrests SNARE complexes until the appropriate calcium signal is provided. Recent approaches using cross-species rescue experiments showed that murine and *Drosophila* complexins have both facilitatory and inhibitory functions associated with similar protein domains in synaptic vesicle exocytosis (10).

Significant alterations of complexins I and II expression levels are seen in a number of neurological and psychiatric disorders, including bipolar disorder (11–13), major depression (12,14), Huntington's disease (HD) (15,16), schizophrenia

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(11,14,17–21), Parkinson's disease (22) and Alzheimer's disease (23). Changes in complexins have also been seen in animal or cell culture models of depression (24), HD (25–27) and Parkinson's disease (28). However, it is not clear whether complexin dysregulation plays a direct role in the aetiology of the disorders in which they have been implicated and contributes to the symptoms, or if they are compensatory.

The importance of complexins is unequivocal since double knockout mice die at birth (3). The two major isoforms of complexin are differentially and reciprocally distributed in the brain (29,30). The highest expression levels of *Cplx1* are seen in the cortex, thalamus and deep cerebellar nuclei. In contrast, *Cplx2* had highest expression in the striatum, hippocampus, amygdaloid nuclei and cortex.

As might be expected from their expression patterns, the behavioural phenotypes of the single knockouts are very different. *Cplx 1* knockout ($Cplx1^{-/-}$) mice exhibit early neurological symptoms characterized by a profound ataxia (present from postnatal day 7 and represents the earliest known ataxia in a mouse model; 31,32). In addition to their pronounced locomotor deficits, $Cplx1^{-/-}$ mice also have deficits in many aspects of social behaviour and tasks reflecting emotional reactivity, although they appear to have normal cognitive function (33). In contrast, adult *Cplx2* knockout ($Cplx2^{-/-}$) mice appear outwardly normal. Although their behavioural abnormalities are effectively 'hidden', comprehensive behavioural testing revealed subtle underlying deficits in motor function while cognitive function is significantly impaired in $Cplx2^{-/-}$ mice (34). $Cplx2^{-/-}$ mice have learning deficits in the Morris water maze (MWM, both acquisition and reversal) and the two-choice swim tank (reversal). The reversal learning deficits are particularly noticeable, being present from the earliest time of testing (8 weeks in the MWM), when most other behaviours are normal. $Cplx2^{-/-}$ mice also fail to develop adult patterns of exploratory behaviour in the open field and show deficits in interactive grooming behaviours (34). Furthermore, they have selective impairments in mossy fibre (MF)-long-term potentiation (LTP) in the CA3 region of the hippocampus of $Cplx2^{-/-}$ mice (35). Synaptic plasticity, such as LTP, is a proposed neural substrate of learning and memory in the hippocampus. This impairment in MF-LTP could contribute to the spatial learning deficits observed in $Cplx2^{-/-}$ mice. 'Emotional' deficits have never been tested in $Cplx2^{-/-}$ mice, although this would be predicted since there is high expression of complexin II throughout the limbic system (30), that has long been implicated in the mediation of emotional and social behaviours. Given that emotional/social abnormalities are common in the diseases in which complexin is dysregulated, we hypothesized that absence of complexin II in the limbic system might result in deficits in these behaviours in $Cplx2^{-/-}$ mice.

RESULTS

$Cplx2^{-/-}$ mice show impairments in social interaction that are reversed by clorgyline

$Cplx2^{-/-}$ mice show impairments in social recognition and memory compared with $Cplx2^{+/+}$mice (Fig. 1) that were reversed by 3 weeks of clorgyline treatment. Although $Cplx2^{+/+}$ and $Cplx2^{-/-}$ mice showed the expected decline in time spent interacting with visitor 1 across successive trials (trial, $F_{3,102} = 12.1851$, $P < 0.001$ for male visitors; $F_{3,102} = 12.0856$, $P < 0.001$ for female visitors), $Cplx2^{-/-}$ mice spent less time interacting than $Cplx2^{+/+}$mice (Fig. 1C and D, genotype, $F_{1,34} = 25.9089$, $P < 0.001$ for male visitors). All mice, regardless of genotype, spent more time investigating female mice than juvenile male mice (Fig. 1D). However, $Cplx2^{-/-}$ mice spent significantly less time investigating female mice than $Cplx2^{+/+}$mice (50% in $Cplx2^{+/+}$ mice compared with 33% in $Cplx2^{-/-}$ mice during trial 1; genotype, $F_{1,36} = 19.6862$, $P < 0.001$).

Clorgyline-treatment reversed the deficits seen in $Cplx2^{-/-}$ mice (Fig. 1E–H). Vehicle-treated $Cplx2^{+/+}$ mice (55%), clorgyline-treated $Cplx2^{-/-}$ mice (36%) and $Cplx2^{+/+}$mice (49%) all spent significantly more time interacting with a novel male visitor compared with vehicle-treated $Cplx2^{-/-}$ mice (17%) (percentages given for trial 1). While clorgyline-treated $Cplx2^{-/-}$ mice and $Cplx2^{+/+}$ mice showed the expected decline in time spent interacting with visitor 1 with each successive trial (Fig. 1G and H), vehicle-treated $Cplx2^{-/-}$ mice showed consistently lower levels of interaction with a novel male visitor mouse similar to the results obtained from the pre-treated $Cplx2^{-/-}$ mice (Fig. 1C and D, trial × genotype × treatment, $F_{3,96} = 3.1705$, $P < 0.05$ for male visitors). There was no significant trial × genotype × treatment interaction for time spent interacting during the first four trials with female visitors.

During the second part of the test, when a second novel mouse (visitor 2) was introduced, the initial high level of social investigation was restored in $Cplx2^{+/+}$mice. However, $Cplx2^{-/-}$ mice failed to investigate the novel mouse during trial 5 irrespective of the sex of the visitor mouse (Fig. 1C and D, $U = 42$, $P < 0.0001$, male visitor; $U = 13$, $P < 0.0001$, female visitor). $Cplx2^{+/+}$mice spent three times longer than $Cplx2^{-/-}$ mice interacting with a novel male mouse and twice as long interacting with a novel female mouse during trial 5. Thus, in addition to the lack of interest in a novel mouse, $Cplx2^{-/-}$ mice failed also to respond to or recognize a second novel mouse.

When treated with clorgyline, $Cplx2^{-/-}$ mice spent significantly more time (34%) investigating a novel male mouse during trial 5 (Fig. 1G, Kruskal–Wallis statistic = 12.5, $P < 0.01$) than vehicle-treated $Cplx2^{-/-}$ mice (13%, Fig. 1E). Furthermore, there were no significant differences between clorgyline-treated $Cplx2^{-/-}$ mice, clorgyline-treated $Cplx2^{+/+}$mice (37%, Fig. 1G) and vehicle-treated $Cplx2^{+/+}$mice (40%, Fig. 1E). Similar differences were found using female visitor mice (Fig. 1F and H, Kruskal–Wallis statistic = 12.5, $P < 0.01$).

Clorgyline-treatment improves performance in the forced swim test in $Cplx2^{-/-}$ mice

$Cplx2^{-/-}$ mice exhibit depressive behaviour in the forced swim test. $Cplx2^{-/-}$ mice were quicker to start floating than $Cplx^{+/+}$mice ($U = 22$, $P < 0.0001$) and spent significantly more time floating than $Cplx^{+/+}$mice ($U = 2$, $P < 0.0001$, data not shown). Both of these deficits improved with clorgyline treatment (Fig. 2A and B, Kruskal–Wallis

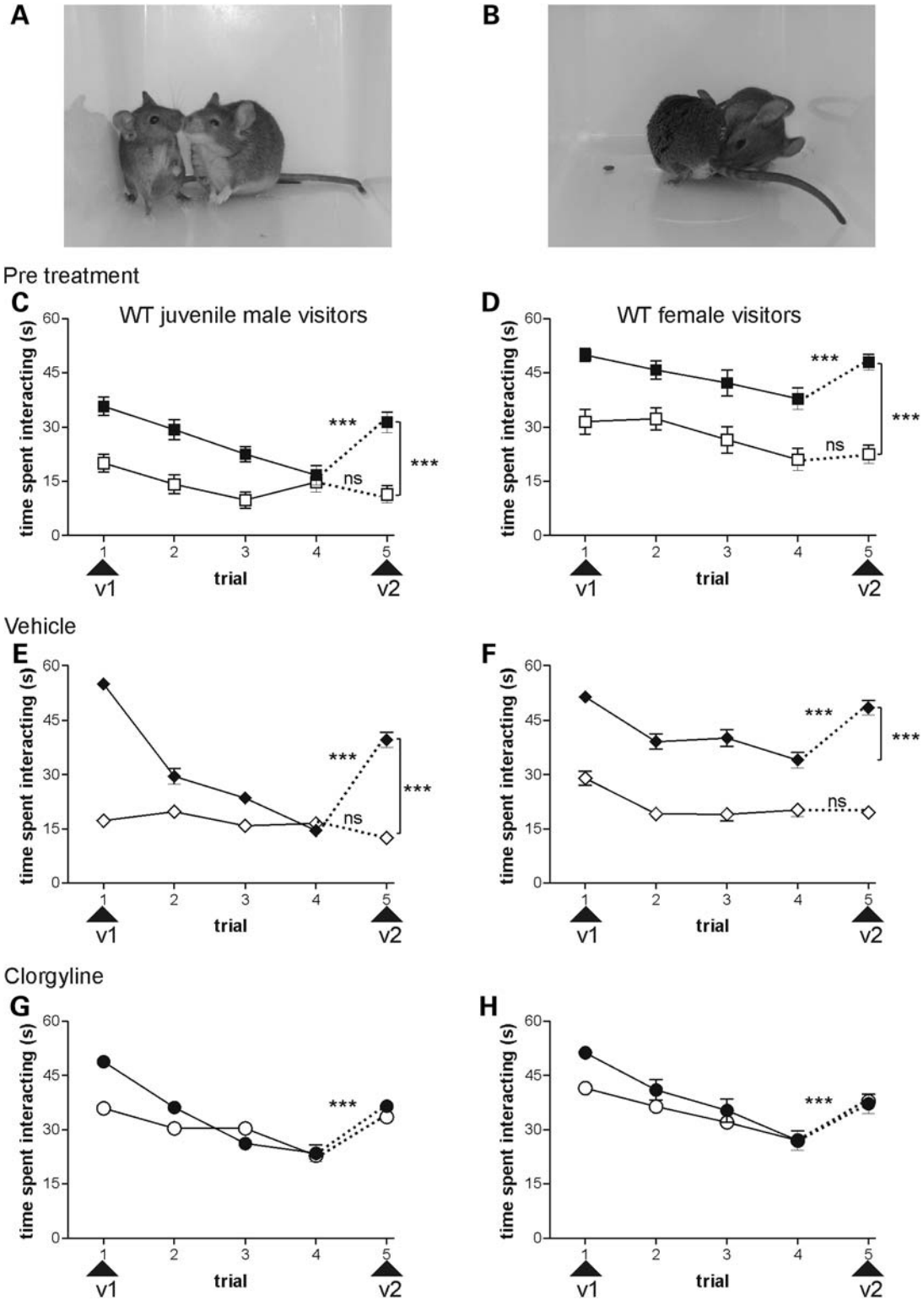

Figure 1. *Cplx2*$^{-/-}$ mice have deficits in social recognition that are reversed with clorgyline treatment at 4–5 months of age. A resident male mouse investigates the 'visitor' mouse by sniffing the nape or muzzle of the 'visitor' mouse (**A**) or by anogenital sniffing (**B**). The time spent interacting with novel male (**C**) and female (**D**) visitor mice by *Cplx*$^{+/+}$mice (black squares) and *Cplx2*$^{-/-}$ mice (white squares) was measured and these mice were then randomly assigned to either vehicle (**E** and **F**, black diamonds = *Cplx*$^{+/+}$ mice, white diamonds = *Cplx2*$^{-/-}$ mice) or clorgyline (**G** and **H**, black circles = *Cplx*$^{+/+}$ mice, white circles = *Cplx2*$^{-/-}$ mice) groups, and retested after 4 weeks clorgyline treatment. Symbols show the mean ± SEM of time spent by each group for interacting with the visitor mouse. Where error bars are not visible, they are obscured by the symbols. Juvenile male (C, E and G) and female (D, F and H) mice were used as 'visitor' mice (black triangles). V1, visitor 1; V2, visitor 2. Asterisks indicate a significant difference (***$P < 0.001$ between *Cplx*$^{+/+}$ and *Cplx2*$^{-/-}$ mice in trial 5 and between trials 4 and 5 within genotype groups).

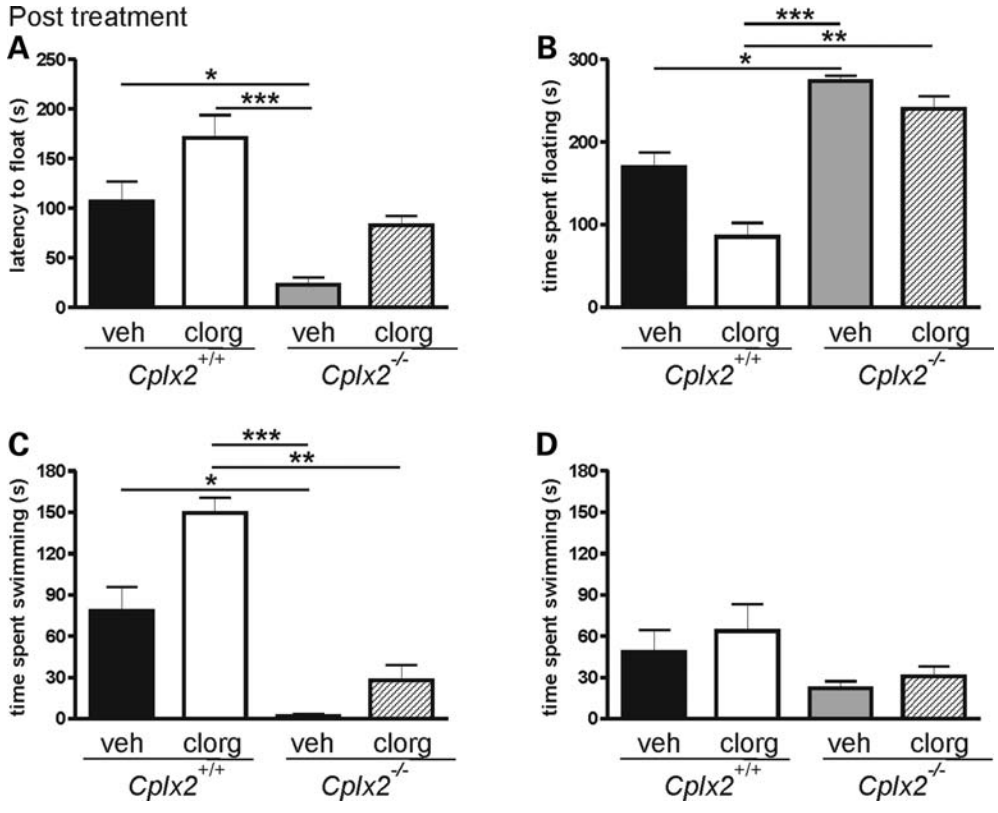

Figure 2. *Cplx2*$^{-/-}$ mice show depressive behaviour in the forced swim test that is ameliorated by clorgyline treatment. Latency to start floating (after 30 s, **A**) and total time spent floating (after 120 s, **B**) were measured in vehicle-treated (filled bars, *Cplx*$^{+/+}$ mice; grey bars, *Cplx2*$^{-/-}$ mice) and clorgyline-treated (open bars, *Cplx*$^{+/+}$ mice; hatched bars, *Cplx2*$^{-/-}$ mice) mice. Swimming behaviour was classified as either time spent vertically (**C**) or horizontally swimming (**D**). Bars show the mean ± SEM of each group on each measure. Asterisks indicate a significant difference ($^{*}P < 0.05$, $^{***}P < 0.001$).

statistic = 20.75, $P < 0.0001$, latency; Kruskal–Wallis statistic = 24.87, $P < 0.0001$, time spent floating). Interestingly, clorgyline-treated *Cplx2*$^{-/-}$ mice spent more time trying to escape the water (vertical swimming) than vehicle-treated *Cplx2*$^{-/-}$ mice (Fig. 2C, Kruskal-Wallis statistic = 24.77, $P < 0.0001$).

Clorgyline-treatment improves locomotor and exploratory deficits in *Cplx2*$^{-/-}$ mice

Cplx2$^{-/-}$ mice have locomotor and exploratory deficits in the open field (34). Here we used an automated system; the Laboratory Animal Behaviour Observation Registration and Analysis System (LABORAS) to examine locomotor and exploratory behaviours in *Cplx2* mice. *Cplx2*$^{-/-}$ mice exhibited impairments in exploration (Fig. 3, Supplementary Material, Fig. S1), showing significantly lower levels of climbing activity (Fig. 3A, genotype, $F_{1,33} = 12.32$, $P < 0.001$) and reduced motor activity (Fig. 3B, genotype, $F_{1,33} = 6.07$, $P < 0.05$) than *Cplx*$^{+/+}$mice. Consequently, *Cplx2*$^{-/-}$ mice show an increased immobility (Fig. 3C, genotype, $F_{1,33} = 4.28$, $P < 0.05$) and travel at a slower speed (Fig. 3D, genotype, $F_{1,33} = 12.14$, $P < 0.001$) than *Cplx*$^{+/+}$ mice.

After clorgyline treatment, deficits improved in three out of the four aspects of locomotor and exploratory behaviours in *Cplx2*$^{-/-}$ mice (Fig. 4, Supplementary Material, Fig. S2). Clorgyline treatment had a main effect on the incidence of climbing (Fig. 4A, $F_{3,31} = 16.96$, $P < 0.01$), significantly increased levels of motor activity (Fig. 4B, $F_{3,31} = 11.13$, $P < 0.01$) and locomotor speed (Fig. 4C, $F_{3,31} = 6.31$, $P < 0.05$) in *Cplx2*$^{-/-}$ mice, while reducing the amount of time the mice spent immobile (Fig. 4D, $F_{3,31} = 13.55$, $P < 0.001$).

Cplx2$^{-/-}$ mice show reductions in noradrenaline, homovanillic acid and 5-hydroxyindoleacetic acid from 2 months of age

There were relatively few changes seen in the neurochemistry of *Cplx2*$^{-/-}$ mice. Dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxytryptamine/serotonin (5-HT) levels in the hippocampus increased significantly between 2 and 8 months, but these increases were independent of genotype ($P < 0.001$, Table 1). DA and homovanillic acid (HVA) levels in the midbrain decreased significantly over the same time period, again independent of genotype ($P < 0.001$, Table 1).

However, significant reductions of noradrenaline (NA) were found in the hippocampus of *Cplx2*$^{-/-}$ mice compared with *Cplx*$^{+/+}$ mice (Table 1, $P < 0.001$) from 2 months of age. HVA, a major metabolite of dopamine, was also reduced in the midbrain ($P < 0.01$) and 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, was reduced in the hippocampus and midbrain ($P < 0.001$ for both regions). No differences in any measurements were found in the stria-

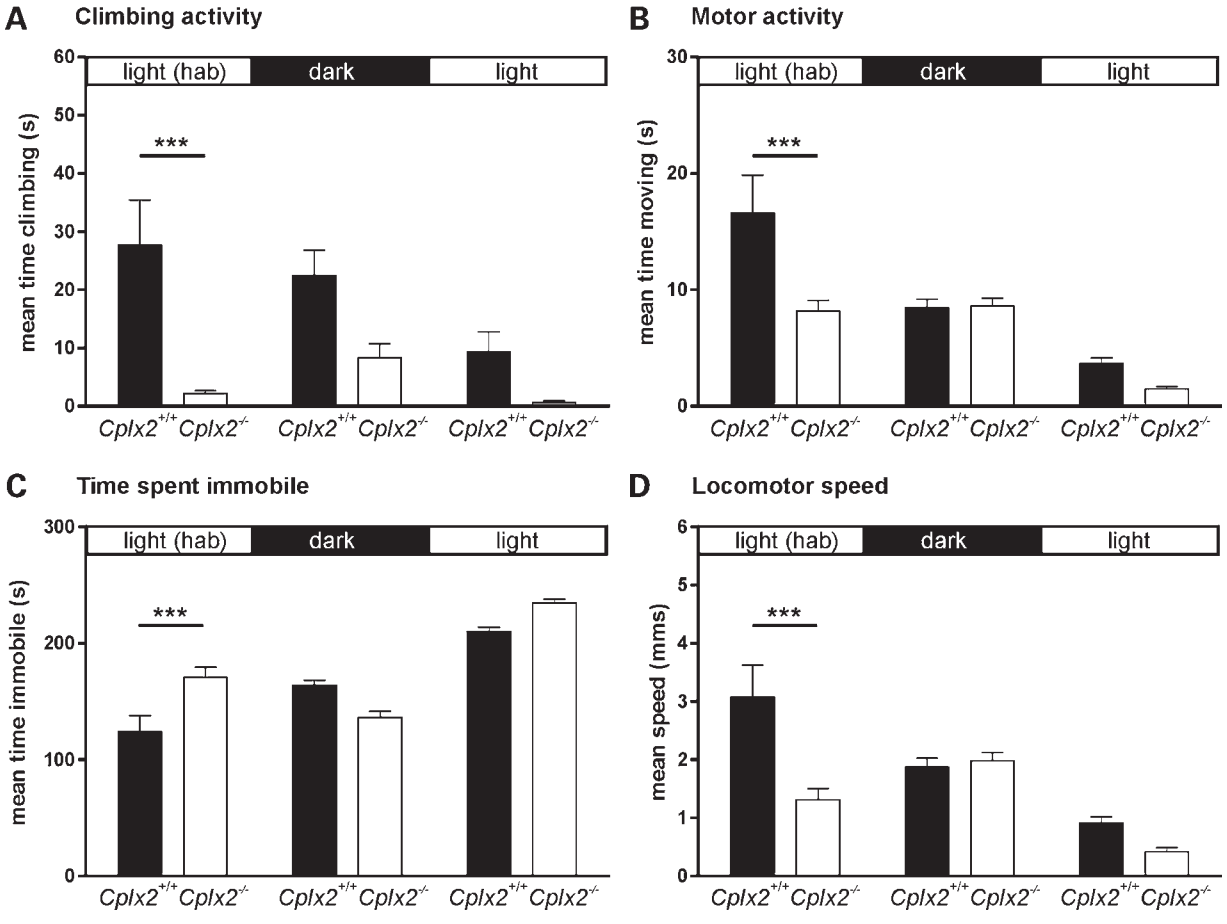

Figure 3. *Cplx2*$^{-/-}$ mice have deficits in locomotion and exploration as measured in the LABORAS behavioural system. The LABORAS was used to measure climbing activity (**A**), motor activity (**B**), time spent immobile (**C**) and locomotor speed (**D**) in *Cplx*$^{+/+}$ (filled bars) and *Cplx2*$^{-/-}$ mice (open bars). Bars show the mean ± SEM of each group on each measure in 5-min bins. Asterisks indicate a significant difference (***$P < 0.001$).

tum, cerebellum and frontal cortex of *Cplx2*$^{-/-}$ mouse brains at 8 months (Supplementary Material, Table S1).

Clorgyline treatment restores NA levels in the hippocampus of *Cplx2*$^{-/-}$ mice

Clorgyline treatment reversed NA deficits in the hippocampus (Table 2, clorgyline, $P < 0.001$, genotype, $P < 0.001$) of *Cplx2*$^{-/-}$ mice to those seen in vehicle-treated *Cplx*$^{+/+}$ mice. No other genotype-specific effects of clorgyline were seen. Clorgyline also had effects on other neurotransmitters, but these were not genotype dependent (Supplementary Material, Table S2).

Abnormalities in *Cplx2*$^{-/-}$ MF-LTP are reversed by isoprenaline

We have previously reported that MF-LTP is significantly reduced in *Cplx2*$^{-/-}$ mice (35). The noradrenergic agonist, isoprenaline (1 μM) partially restored MF-LTP to 143 ± 3% ($n = 6$) in *Cplx2*$^{-/-}$ mice (Fig. 5A and B, $F_{3,42} = 99.84$, $P < 0.0001$) but had no significant effect on MF-LTP in *Cplx*$^{+/+}$mice (Fig. 5B, 191 ± 6%, $n = 5$). In hippocampal cell cultures from mice lacking complexins I and II, the Ca^{2+}-sensitivity of glutamate release is decreased (3). One effect of isoprenaline in dentate gyrus granule cells, the source of MFs, is to enhance the voltage-gated Ca^{2+}current (36). We induced MF-LTP in the presence of elevated concentrations of extracellular Ca^{2+}. For example, in the presence of 5 mM Ca^{2+}, MF-LTP was partially restored in *Cplx2*$^{-/-}$ mice (Fig. 5C and D, 127 ± 2%, $n = 6$; $F_{4,47} = 84.3$, $P < 0.0001$). In the combined presence of elevated Ca^{2+} (5 mM) and isoprenaline (1 μM), MF-LTP in *Cplx2*$^{-/-}$ mice was completely restored to control levels (Fig. 5E and F, 208 ± 12%, $n = 5$; $F_{2,37} = 127.4$, $P < 0.0001$).

DISCUSSION

We have shown previously that although *Cplx2*$^{-/-}$ mice appear outwardly normal, they exhibit progressive deficits in motor and cognitive behaviours from an early age, with the precise age of onset dependent upon the particular test (34). In addition, they have abnormalities in synaptic plasticity that might contribute to these deficits (35). Here we show that *Cplx2*$^{-/-}$ mice also have significant deficits in social recognition and social interactions, and they show depressive-like behaviours. Pharmacological treatment with clorgyline reverses multiple aspects of the abnormal phenotype in

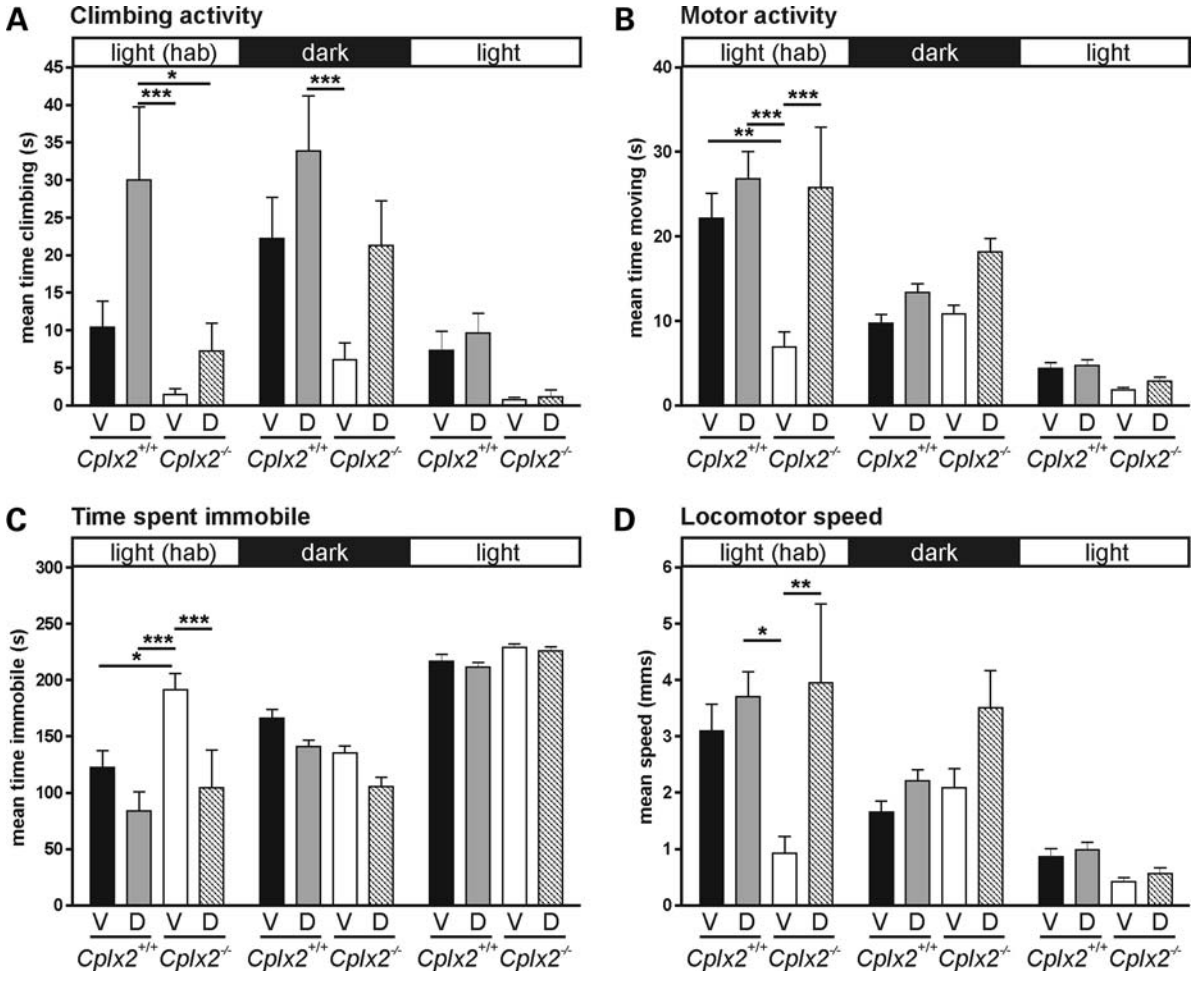

Figure 4. The effects of clorgyline treatment were assessed on climbing activity (**A**), motor activity (**B**), time spent immobile (**C**) and locomotor speed (**D**) in vehicle-treated (filled bars, $Cplx^{+/+}$ mice; open bars, $Cplx2^{-/-}$ mice) and clorgyline-treated (grey bars, $Cplx^{+/+}$ mice; hatched bars, $Cplx2^{-/-}$ mice) mice. Bars show the mean ± SEM of each group on each measure in 5-min bins. Asterisks indicate a significant difference ($^{*}P < 0.05$, $^{**}P < 0.01$ $^{***}P < 0.001$).

Table 1. Neurochemical measurements in *Cplx2* mouse brain (ng/g tissue ± SEM)

	Genotype	NA	DA	DOPAC	HVA	5HT	5-HIAA
Midbrain							
2 months	$Cplx^{+/+}$	705 ± 61	1845 ± 175	502 ± 29	465 ± 25	424 ± 27	408 ± 22
	$Cplx2^{-/-}$	666 ± 45	1715 ± 184	413 ± 52	342 ± 26 ↓	445 ± 36	331 ± 18 ↓
8 months	$Cplx^{+/+}$	668 ± 59	911 ± 92	362 ± 22	260 ± 18	473 ± 37	377 ± 25
	$Cplx2^{-/-}$	633 ± 19	1003 ± 141	393 ± 26	247 ± 18	497 ± 51	275 ± 19 ↓
Time		$P = 0.474$	**$P < 0.001$**	**$P < 0.001$**	**$P < 0.001$**	$P = 0.210$	$P = 0.06$
Genotype		$P = 0.446$	$P = 0.905$	$P = 0.222$	**$P < 0.01$**	$P = 0.571$	**$P < 0.001$**
Time × genotype		$P = 0.972$	$P = 0.484$	$P = 0.985$	**$P < 0.05$**	$P = 0.972$	$P = 0.573$
Hippocampus							
2 months	$Cplx^{+/+}$	327 ± 17	49 ± 4	40 ± 2	60 ± 4	397 ± 39	384 ± 23
	$Cplx2^{-/-}$	195 ± 15 ↓	46 ± 2	31 ± 2	62 ± 6	400 ± 23	317 ± 21 ↓
8 months	$Cplx^{+/+}$	270 ± 6	87 ± 10	208 ± 9	77 ± 13	521 ± 33	377 ± 19
	$Cplx2^{-/-}$	203 ± 12 ↓	77 ± 6	199 ± 10	69 ± 9	533 ± 32	275 ± 25 ↓
Time		$P = 0.088$	**$P < 0.001$**	**$P < 0.001$**	$P = 0.218$	**$P < 0.001$**	$P = 0.285$
Genotype		**$P < 0.001$**	$P = 0.095$	$P = 0.222$	$P = 0.714$	$P = 0.769$	**$P < 0.001$**
Time × genotype		**$P < 0.05$**	$P = 0.240$	$P = 0.985$	$P = 0.605$	$P = 0.954$	$P = 0.452$

Significant results are shown in bold.

$Cplx2^{-/-}$ mice. To our knowledge, this is the first evidence of pharmacological reversal of behavioural deficits in a mouse in which synaptic dysfunction has been caused by gene knockout. It is possible that deficits in sensory function in $Cplx2^{-/-}$ mice may have contributed to the abnormal behaviours reported here. We have not measured sensory function

Table 2. Neurochemical measurements in *Cplx2* mouse brain post treatment (ng/g tissue ± SEM)

	Genotype		NA	DA	DOPAC	HVA	5HT	5-HIAA
Midbrain								
	$Cplx^{+/+}$	Vehicle	508 ± 35	768 ± 121	341 ± 47	139 ± 7	514 ± 67	323 ± 19
	$Cplx^{+/+}$	Clorgyline	817 ± 65 ↑	1090 ± 155 ↑	224 ± 23	88 ± 5.4	1039 ± 44 ↑	236 ± 33↓
	$Cplx2^{-/-}$	Vehicle	519 ± 40	404 ± 118	242 ± 42	106 ± 14	453 ± 21	312 ± 31
	$Cplx2^{-/-}$	Clorgyline	902 ± 89 ↑	1040 ± 141 ↑	312 ± 36	89 ± 8	1085 ± 60 ↑	237 ± 39↓
Genotype			$P = 0.458$	$P = 0.112$	$P = 0.188$	$P = 0.448$	$P = 0.888$	$P = 0.852$
Clorgyline			***P* < 0.001**	***P* < 0.001**	$P = 0.604$	$P = 0.644$	***P* < 0.001**	***P* < 0.05**
Clorgyline × genotype			$P = 0.566$	$P = 0.211$	$P = 0.134$	$P = 0.974$	$P = 0.295$	$P = 0.906$
Hippocampus								
	$Cplx^{+/+}$	Vehicle	370 ± 17	75 ± 14	56 ± 6	83 ± 6	232 ± 12	309 ± 28
	$Cplx^{+/+}$	Clorgyline	456 ± 14 ↑	92 ± 22	38 ± 3	60 ± 8	417 ± 16 ↑	252 ± 22 ↓
	$Cplx2^{-/-}$	Vehicle	280 ± 16	60 ± 13	44 ± 3	85 ± 17	265 ± 13	298 ± 24
	$Cplx2^{-/-}$	Clorgyline	360 ± 17 ↑	60 ± 16	51 ± 6	67 ± 10	429 ± 26 ↑	217 ± 24 ↓
Genotype			***P* < 0.001**	$P = 0.192$	$P = 0.831$	$P = 0.688$	$P = 0.233$	$P = 0.357$
Clorgyline			***P* < 0.001**	$P = 0.625$	$P = 0.273$	$P = 0.077$	***P* < 0.001**	***P* < 0.01**
Clorgyline × genotype			$P = 0.835$	$P = 0.634$	***P* < 0.05**	$P = 0.807$	$P = 0.575$	$P = 0.64$

Significant results are shown in bold.

directly. However, there are no major deficits in vision, since $Cplx2^{-/-}$ mice can find a visible platform as easily as wild-type (WT) mice (34). We have also shown in preliminary studies that $Cplx2^{-/-}$ mice aged <1 year have a normal acoustic startle response (D. Glynn and A. Jennifer Morton unpublished observations) and are capable of distinguishing between flavoured and normal chow (Supplementary Material, Fig. S3), suggesting that there are no major deficits in hearing or olfactory discrimination. Therefore, we suggest that the deficits reported here were not mediated by the changes in sensory function although we cannot rule out a contribution from sensory impairment.

Clorgyline treatment reversed the decrease in levels of NA found in the hippocampus. Clorgyline is an irreversible inhibitor of monoamine oxidase (MAO) that was developed originally as an antidepressant but is no longer used in humans. It increases NA levels, although its effects are not selective and it also increases the levels of serotonin, adrenaline and dopamine. There is evidence to suggest that restoration of NA levels by clorgyline may underlie the improvements in behaviour seen here in $Cplx2^{-/-}$ mice. First, in the forced swim test, it has been shown that climbing/escape swimming behaviour was increased by antidepressant drugs with NA selective effects whereas horizontal swimming behaviour was increased by selective serotonin reuptake inhibitors (SSRIs, 5-HT dependent; 37). Here, clorgyline-treated $Cplx2^{-/-}$ mice spent more time engaged in climbing/escape swimming behaviours than vehicle-treated $Cplx2^{-/-}$ mice, whereas there was no difference in horizontal swimming behaviour (5-HT dependent) between any of the groups. It is possible that the increase in swimming behaviour observed was a result of a general increase in locomotor activity caused by the drug rather than by its 'antidepressant' action. However, we have shown previously that swimming style and strength is normal in $Cplx2^{-/-}$ mice, and furthermore that there was no significant differences in swimspeed between $Cplx2^{-/-}$ mice and WT controls at 1 year in the MWM (34). Therefore, we conclude that this specific increase in climbing/escape swimming behaviour suggests that the improvements were mediated by the antidepressant action of the drug rather than a general increase in locomotor activity. Secondly, activity is proportional to concentrations of NA. There is evidence that an increased investigative activity and a decreased emotionality in a novel environment are seen in animals with higher levels of NA synthesis and a decreased density of adrenergic receptors in the brain (38). Here, we show that $Cplx2^{-/-}$ mice exhibit their most profound deficits in motor and exploratory behaviour in the LABORAS during the first light period when the mice are habituating to a novel environment. Of particular interest is that clorgyline treatment reversed these deficits during this time period in $Cplx2^{-/-}$ mice. Note that although we used clorgyline for our study, this would not be suitable for use in humans. Side effects (in particular, the 'cheese effect') have limited the use of irreversible MAO inhibitors as therapeutic agents (39). These deleterious side effects can now be avoided by using reversible MAOA inhibitors such as moclobemide and lazabemide or other new generation antidepressant agents (39).

We were able to reverse the selective deficit seen in LTP in $Cplx2^{-/-}$ mice using isoprenaline. Isoprenaline is a β_1- and β_2-adrenoceptor agonist and as such mimics the action of NA. It is possible that NA reduction may underlie the LTP deficit in $Cplx2^{-/-}$ mice. Noradrenergic enhancement of LTP in mossy fibre synapses in the hippocampus has been shown previously (40–42). Activity-dependent alterations in synaptic efficacy (synaptic plasticity), such as LTP and long-term depression (LTD), are widely believed to underlie information processing and storage in the brain (43). Reduced plasticity is evident in the hippocampus of $Cplx2^{-/-}$ mice weeks before the first signs of an overt phenotype in $Cplx2^{-/-}$ mice, thus supporting the suggestion that altered synaptic plasticity contributes to the cognitive dysfunction seen in these mice particularly in the early stages of their phenotype. It would be interesting to investigate whether clorgyline treatment would also reverse the deficits in learning and memory in the MWM that are seen in $Cplx2^{-/-}$ mice, since the MWM is a task commonly used to test spatial memory, and accurate performance depends on intact hippocampal function (44).

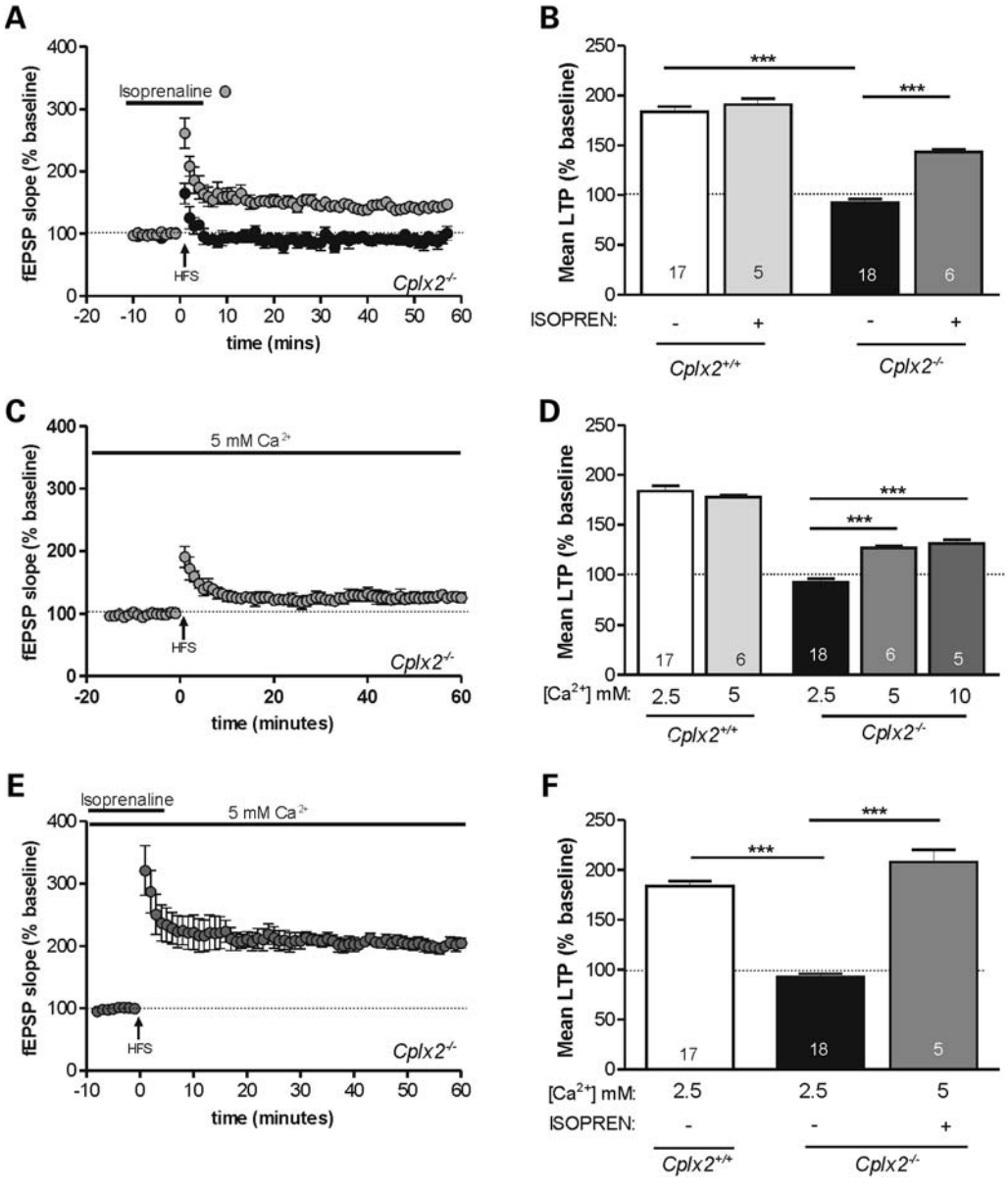

Figure 5. Abnormalities in *Cplx2*$^{-/-}$ MF-LTP can be reversed in the presence of isoprenaline and elevated extracellular Ca^{2+}. Normalized MF-fEPSPs in *Cplx2*$^{-/-}$ mice in the presence or absence of 1 μM isoprenaline (**A**). The bar graph summarizes LTP in slices from *Cplx*$^{+/+}$ or *Cplx2*$^{-/-}$ mice in the absence or presence of 1 μM isoprenaline. Isoprenaline caused a significant increase in LTP in *Cplx2*$^{-/-}$ mice (**B**). Normalized MF-fEPSPs in *Cplx2*$^{-/-}$ mice in the presence of 5 mM extracellular Ca^{2+} (**C**). LTP in slices from *Cplx*$^{+/+}$ or *Cplx2*$^{-/-}$ mice in elevated extracellular Ca^{2+} (**D**). Normalized MF-fEPSPs in five *Cplx2*$^{-/-}$ mice in 1 μM isoprenaline and 5 mM extracellular Ca^{2+} (**E**). LTP in the presence of 1 μM isoprenaline plus 5 mM extracellular Ca^{2+} (**F**). Bars show the mean ± SEM of each group on each measure. Asterisks indicate a significant difference (***$P < 0.001$).

There is a growing body of evidence to support the idea that disturbed synaptic transmission contributes to the pathophysiology of mood disorders (45–48), although it is not clear whether dysregulated complexin expression plays a causal role or whether it contributes solely to the symptomatology (reviewed by 49). Post-mortem studies have reported reduced complexin expression levels in bipolar disorder and major depression (11–14) and in an animal model of depression (24). Zink *et al.* (50) have also shown that antidepressant treatment with desipramine (reuptake inhibitor of NA) and tranylcypromine (MAOA/B inhibitor) increases *Cplx2* mRNA expression in WT mice, whereas fluoxetine (SSRI) had no effect on *Cplx2* expression (although it did increase *Cplx1* expression).

Here, we found significant reductions of NA in the hippocampus of *Cplx2*$^{-/-}$ mice that were reversed after clorgyline treatment. We did not measure mRNA expression, although based on the findings of Zink *et al.* it seems likely that upregulation of complexin as a result of selective enhancement of NA might facilitate SNARE complex function and synaptic transmission at the synapse (50).

The phenotype of the *Cplx2*$^{-/-}$ mouse recapitulates many of the subtle aspects or endophenotypes of neurological disease (e.g. deficits in cognitive and social behaviour,

depression and subtle motor abnormalities). Here we have shown that pharmacological treatment with clorgyline can reverse deficits in neurochemistry, behaviour and electrophysiology in *Cplx2*$^{-/-}$ mice. These data support the idea that *Cplx2* is an attractive candidate gene involved in the fundamental processes of affective disorders and as such an important possible target for antidepressive therapy.

MATERIALS AND METHODS

Animals

Cplx2 mice were generated by homologous recombination in embryonic stem cells (3). All mice used in this study were F1 or F2 mice bred from founders with a mixed genetic background (129Ola/C57Bl6) and were taken from a colony established in the Department of Pharmacology, University of Cambridge. *Cplx2*$^{-/-}$ mice have now been backcrossed onto a C57/Bl6 inbred background for 10 generations, but no change in their overt or measured phenotype has been observed (data not shown). Mice were housed and husbandry maintained as described previously (34). In addition, all mice had an environment enriched by the presence of a red Perspex igloo (Datesand, Manchester, UK) in their home cage. All experimental procedures were licensed and undertaken in accordance with the regulations of the UK Animals (Scientific Procedures) Act 1986.

Drug treatment

All mice were dosed daily by intraperitoneal (ip) injection with either clorgyline (Sigma, UK) or vehicle (0.9% saline). Clorgyline was administered at a dose of 1.5 mg/kg. Mice were treated for at least 21 days prior to post-drug behavioural testing and treatment continued until the end of the experiment. Specific details (ages, genotypes and group sizes) of mice used are detailed below.

Social recognition

Social interactions (recognition and memory) were measured in a test described by Ferguson *et al*. (51). The task is based on the natural tendency of WT mice to investigate another mouse that is introduced to its home cage. When the same 'visitor' mouse is presented repeatedly, the response of the resident mouse declines to a low level. If a new 'visitor' mouse is then presented, the initial level of social investigation is typically reinstated. Both juvenile male and female mice were used as 'visitor' mice. Briefly, a 'resident' mouse was placed in a clean cage and allowed habituate for 10 min, after which an unfamiliar mouse (visitor 1) was introduced into the cage. After 1 min, visitor 1 was removed, placed in a holding cage and then reintroduced to the resident mouse for a further three 1-min trials at 10 min inter-trial intervals. In the fifth trial, a different unfamiliar mouse (visitor 2) was introduced into the cage. The time the resident mouse spent interacting with each visitor mouse was recorded. Mice were tested at 4 months of age (16 *Cplx*$^{+/+}$, 19 *Cplx2*$^{-/-}$). These mice were then randomly assigned to either drug (8 *Cplx*$^{+/+}$, 9 *Cplx2*$^{-/-}$) or vehicle (8 *Cplx*$^{+/+}$, 10 *Cplx2*$^{-/-}$) groups, and retested after 4 weeks clorgyline treatment.

Forced swim test

Depressive behaviour was tested in the forced swim test, a standard animal test of depression used to demonstrate the efficacy of antidepressants (52). Each mouse was placed into water 22 cm deep at ambient temperature (21 $\pm$ 1°C) in a 5 l Pyrex beaker for 7 min. The following parameters were measured: the latency to float (after the first 30 s); the duration of immobility time (after the first 120 s, measured when the mouse floated in the water in an upright position, making only slight movements to keep its head above the surface); climbing behaviour (upward directed movements of the forepaws); horizontal swimming behaviour. *Cplx2* mice were tested at 1 year (18 *Cplx*$^{+/+}$, 16 *Cplx2*$^{-/-}$) and were then randomly assigned to either drug (9 *Cplx*$^{+/+}$, 7 *Cplx2*$^{-/-}$) or vehicle (9 *Cplx*$^{+/+}$, 9 *Cplx2*$^{-/-}$) groups, and retested after 3 weeks clorgyline treatment.

Motor activity

The LABORAS (Metris b.v., The Netherlands) was used to measure motor activity and exploration (53,54). Mice were singly housed with *ad libitum* access to food and water and monitored for 23.5 h. Mice were tested at 4 months (16 *Cplx*$^{+/+}$, 19 *Cplx2*$^{-/-}$), after which they were randomly assigned to either drug (8 *Cplx*$^{+/+}$, 9 *Cplx2*$^{-/-}$) or vehicle (8 *Cplx*$^{+/+}$, 10 *Cplx2*$^{-/-}$) groups, and retested in the LABORAS after 5 weeks clorgyline treatment.

Neurochemistry

Neurochemical measurements were made from five different brain regions (hippocampus, frontal cortex, striatum, cerebellum and midbrain) using high-performance liquid chromatography. NA, DA, DOPAC, HVA, 5-HIAA and 5-HT were measured in all regions. Brain tissue was homogenized and proteins precipitated in 0.1 M perchloric acid and 0.1 mM ascorbic acid. Supernatants were then injected onto a Hypersil 5 μm ODS C18 (150 × 4.6 mm) column and analysed by electrochemical detection. The mobile phase (pH 3.0) consisted of 0.1 M phosphate buffer containing 2.5 mM octane sulphonate, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 20.5 ml acetic acid and 12% methanol, at a flow rate of 1 ml/min. Neurochemical measurements were taken from three different groups of mice; 2 months (8 *Cplx2*$^{-/-}$, 8 *Cplx*$^{+/+}$); 8 months (8 *Cplx2*$^{-/-}$, 8 *Cplx*$^{+/+}$) and 8 months after 7 weeks clorgyline treatment (4 *Cplx2*$^{-/-}$, 4 *Cplx*$^{+/+}$) or vehicle (4 *Cplx2*$^{-/-}$, 8 *Cplx*$^{+/+}$).

Electrophysiology

Experiments were carried out as described in Gibson *et al*. (35). Briefly, hippocampal slices prepared from 4- to 10-week-old male *Cplx*$^{+/+}$ and *Cplx2*$^{-/-}$ mice were perfused with artificial cerebrospinal fluid (26–28°C) containing the γ-aminobutyric acid type A ($GABA_A$)-receptor antagonist, picrotoxin (50 μM). Electrical stimulation at the dentate gyrus granule cell/hilus border evoked MF field excitatory postsynaptic potentials (EPSPs) in stratum lucidum of CA3 (paired pulse ratio > 1.6 and frequency-dependent facilitation;

35,55). MF-LTP was evoked by high-frequency stimulation (HFS; three 1 s 100 Hz trains every 20 s) in the presence of the *N*-methyl-D-aspartic acid receptor antagonist D-AP5 (50 μM). Isoprenaline (1 μM, Tocris Cookson, Bristol, UK) or elevated extracellular Ca^{2+} (5 mM, 10 mM) were perfused for at least 20 min prior to HFS. To calculate LTP, normalized fEPSP slope values were averaged between 30 and 50 min post-HFS.

Statistical analysis

Most behavioural data were subjected to analysis of variance (ANOVA), with one or two between-subject factors (genotype, treatment) with repeated measures. Where applicable, Bonferroni's *post hoc* tests were used. For data that were not normally distributed, the Kruskal–Wallis test with Dunnet's *post hoc* test was used. An unpaired two-tailed *t*-test (or a Mann–Whitney two-tailed test in the case of non-parametric data) was applied to test the significance of differences between mean values where factorial ANOVA was not required. Statistical analyses were performed using GraphPad Prism (Version 4.0, USA) and Statistica (Version 9.0, StatSoft, Inc., USA). A critical value for significance of $P < 0.05$ was used throughout the study.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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