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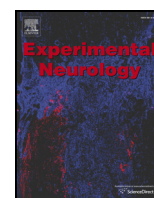
Research Paper

Treatment with the MAO-A inhibitor clorgyline elevates monoamine neurotransmitter levels and improves affective phenotypes in a mouse model of Huntington disease

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ABSTRACT

Abnormal monoamine oxidase A and B (MAO-A/B) activity and an imbalance in monoamine neurotransmitters have been suggested to underlie the pathobiology of depression, a major psychiatric symptom observed in patients with neurodegenerative diseases, such as Huntington disease (HD). Increased MAO-A/B activity has been observed in brain tissue from patients with HD and in human and rodent HD neural cells. Using the YAC128 mouse model of HD, we studied the effect of an irreversible MAO-A inhibitor, clorgyline, on the levels of select monoamine neurotransmitters associated with affective function. We observed a decrease in striatal levels of the MAO-A/B substrates, dopamine and norepinephrine, in YAC128 HD mice compared with wild-type mice, which was accompanied by increased anxiety- and depressive-like behaviour at five months of age. Treatment for 26 days with clorgyline restored dopamine, serotonin, and norepinephrine neurotransmitter levels in the striatum and reduced anxiety- and depressive-like behaviour in YAC128 HD mice. This study supports a potential therapeutic use for MAO-A inhibitors in the treatment of depression and anxiety in patients with HD.

1. Introduction

Monoamine oxidases (MAO) play an important role in brain function via the metabolic regulation of monoamine neurotransmitters, such as dopamine (DA), norepinephrine (NE), and serotonin (5-HT). Alterations in MAO activity, which are associated with changes in monoamine neurotransmitter levels as well as with the production of toxic reactive oxygen species, have been implicated in the pathobiology of neuropsychiatric and neurodegenerative disorders (Duncan et al., 2012). Interestingly, psychiatric manifestations are a common feature of many neurodegenerative disorders including Parkinson's (PD) and Huntington disease (HD). Indeed, depression is the most prevalent symptom in PD and HD, occurring in approximately 40–60% of patients (Paulsen et al., 2005; Youdim and Bakhle, 2006).

Two isoforms of MAO exist, MAO-A and MAO-B, which share approximately 70% amino acid identity. Both proteins are expressed in most mammalian tissues, associated with the outer membrane of the mitochondria (Finberg, 2014; Youdim et al., 2006); however, the ratio of MAO-A and MAO-B isoforms and the levels of MAO activity vary between regions of the human brain (Youdim et al., 2006). Enzymatically, MAO-A and MAO-B differ in their substrate selectivity. NE and 5-HT are specific substrates for MAO-A, whereas phenylethylamine (PEA) and benzylamine are only degraded by MAO-B. DA is a common substrate for both isozymes (Duncan et al., 2012).

Abnormal MAO-A and MAO-B activity are therefore involved in distinct clinical presentations (Duncan et al., 2012). Dysregulation in MAO-A activity has been implicated in a variety of neuropsychiatric disorders including depression, anxiety, autism, and attention deficit hyperactivity disorder, whereas MAO-B activity, which has been described to increase with ageing, is associated with neurodegenerative disorders, such as PD (Duncan et al., 2012). Moreover, alterations in both MAO-A and B activity have been observed in brain regions that undergo neurodegeneration in HD patients, such as the basal ganglia and the pons (Richards et al., 2011).

Monoamine oxidase inhibitors have long been used for treatment of psychiatric disorders and, more recently, have shown therapeutic benefits in the treatment of neurodegenerative disorders (Youdim and Bakhle, 2006). MAO inhibitors can be classified as reversible or

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irreversible inhibitors of MAO-A, MAO-B, or both (Finberg, 2014; Youdim et al., 2006). Inhibition of MAO-A results in antidepressant and anxiolytic effects (Cryan and Holmes, 2005; Finberg, 2014), whereas selective MAO-B inhibitors are useful in movement disorders such as PD (Finberg, 2014; Youdim and Bakhle, 2006). Although MAO inhibitors have yet to be tested in the treatment of HD, the presence of psychiatric manifestations and increased MAO-A and MAO-B activity in HD patients suggest that MAO inhibitors may be of therapeutic benefit.

Recently, we showed that inhibition of excessive MAO activity in mouse and human HD neural cells using clorgyline, an irreversible MAO-A inhibitor, reduces oxidative stress and improves cellular viability (Ooi et al., 2015). These observations prompted us to evaluate the effects of clorgyline further in the YAC128 (FVB/N) mouse model of HD. The YAC128 HD mice express a full-length human mutant *HTT* transgene and exhibit neuropathological and behavioural phenotypes that mimic symptoms of patients with HD, including affective phenotypes such depressive- and anxiety-like behaviour (Pouladi et al., 2013; Slow et al., 2003). After establishing the appropriate dose of clorgyline in wild-type (WT) FVB/N mice, we sought to determine the effect of MAO-A inhibition on monoamine neurotransmitter levels and affective phenotypes in YAC128 HD mice.

2. Material and methods

2.1. Animals

Three-month-old male FVB/N mice, purchased from InVivos (Singapore), used for the initial clorgyline dosing study, and four-month-old YAC128 HD and littermate wild-type mice were group-housed on a reverse 12-h light/dark cycle. The mice had ad libitum access to water and food throughout the study. Clorgyline hydrochloride (Sigma) was diluted in phosphate buffered saline (PBS) and was delivered by intraperitoneal injection (i.p.) once per day. The diluted drug solution was free of precipitates. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Biological Resource Centre, ASTAR.

2.2. Drug treatment

For the clorgyline dosing study, 3 month old WT mice were divided into four groups of 10 mice each. Three groups received clorgyline at a dose of 0.5, 1.5, or 3 mg/kg and the fourth group received an equivalent volume of PBS. For the YAC128 treatment study (Fig. 1A), three independent cohorts of 4 month old mice were used, giving a total of 17–22 mice per treatment/group. For each cohort, mice were divided

Fig. 1. Schematic representation of the experimental design for the clorgyline dosing study in WT mice and efficacy study in YAC128 HD mice. (A) WT mice were injected intraperitoneally (i.p.) with vehicle (PBS) or clorgyline (0.5, 1.5, or 3 mg/kg) daily for 28 days. (B) WT mice were injected i.p. with vehicle (PBS) only, whereas YAC128 HD mice were injected with either vehicle (PBS) or clorgyline (1.5 mg/kg), daily for 26 days. For both (A) and (B), behavioural tests commenced on day 22 and were carried out in this order: open field (OF), elevated plus maze (EPM), tail suspension test (TST), and the forced swim test (FST). Mice were sacrificed on day 26 or 28 and brain tissue was harvested for biochemical assessments.

into three groups. One group of YAC128 HD mice received clorgyline at a dose of 1.5 mg/kg and two groups, one of WT mice and another one of YAC128 HD mice, received an equivalent volume of PBS (Fig. 1B). Treatments were administered daily before noon at a volume of 10 mL/kg i.p. for 21 days and were continued throughout the behavioural testing phase, which commenced on day 22. During the behavioural testing phase, mice were treated in the afternoon following completion of the corresponding behavioural test. Mice were sacrificed once the behavioural testing was finished at day 26 or 28 (Fig. 1).

2.3. Determination of MAO-A activity in brain tissue

The MAO-Glo Assay System (Promega) was used to measure MAO-A activity. Brain tissue was homogenized in lysis buffer (50 mM TRIS pH 8.0, 150 mM NaCl, 1% Igepal, $1\times$ Roche complete proteinase inhibitor, 0.005 mM ZVAD, and 1 mM phenylmethulsulfonyl fluoride in Milli-Q water). Protein lysates were diluted to 1 mg/mL using lysis buffer. Diluted lysate (25 μL) was incubated with an equal volume of MAO substrate solution (1:25 dilution of provided MAO substrate) for 2 h at room temperature. Luciferin detection reagent (50 μL) was added and luminescence was measured using FLUOstar Omega plate reader (BMG Labtech). Except for the lysis buffer, all solutions and reagents were provided by the MAO-Glo Assay System (Promega).

2.4. Determination of dopamine, norepinephrine, serotonin, and DOPAC levels in brain tissues

Following completion of behavioural testing, mice were sacrificed by carbon dioxide asphyxiation followed by rapid removal of the brains. Brains were micro-dissected on ice and immediately snap-frozen in liquid nitrogen. DA, NE, and 5-HT were determined by Brains On-line (Groningen, Netherlands) using established methods. Briefly, for the preparation of LC–MS samples, 6 mL of 0.5 M perchloric acid was added to each mg of striatal tissue and the samples were homogenized by sonication. The homogenates were centrifuged and the supernatants were stored as brain extracts at −80 °C until analysis. For analysis, an aliquot of internal standard solution was mixed with a diluted aliquot of each brain extract sample. The mixture was centrifuged and the supernatant was transferred to a vial suitable for use in the autosampler. Concentrations of 5-HT, DA, NE, and DOPAC were determined by HPLC with tandem mass spectrometry (MS/MS) detection, using deuterated internal standards of the analytes. For each LC–MS sample, an aliquot was injected onto the HPLC column by an automated sample injector (SIL10-20 AC-HT, Shimadzu, Japan). Chromatographic separation was performed on a SynergiMax column (100×3.0 mm, particle size 2.5 μm) held at a temperature of 35 °C. The mobile phases consisted of A: ultra-purified H_2O + 0.1% formic, and B: acetonitrile: ultra-purified H_2O (70:30) + 0.1% formic acid. Elution of the compounds proceeded using a suitable linear gradient at a flow rate of 0.3 mL/min. The MS analyses were performed using an API 4000 MS/MS system consisting of an API 4000 MS/MS detector and a Turbo Ion Spray interface (Applied Biosystems, the Netherlands). The acquisitions on API 4000 were performed in positive ionisation mode, with optimised settings for the analytes. The instrument was operated in multiple-reaction-monitoring (MRM) mode. Data were calibrated and quantified using the Analyst data system (Applied Biosystems, version 1.6.2, Netherlands). Concentrations in experimental samples were calculated based on the calibration curve in the corresponding matrix.

2.5. Behavioural tests of affective function

All behavioural tests were performed in the morning during the dark phase of the reverse light/dark-cycle.
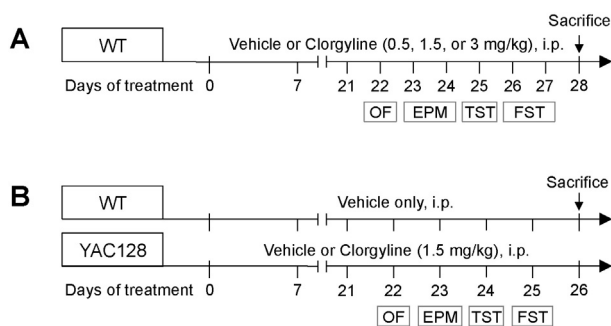
2.5.1. Open-field test of anxiety

The open-field test is commonly used to assess anxiety in rodents (Crawley, 1985). The testing apparatus is a 50 × 50 cm open, grey, acrylic box (open field) with 20-cm high walls. Because rodents have an innate fear of open and bright spaces, they preferentially spend more time at the perimeter rather than the centre of the open field. The time spent in the centre versus the perimeter is taken as a measure of anxiety-like behaviour. Test sessions lasted 10 min and the time spent in the centre versus perimeter was recorded using an automated video-based tracking system (Noldus EthoVision 9, Netherlands).

2.5.2. Elevated plus maze test of anxiety

The Elevated Plus Maze (Walf and Frye, 2007) is a well-established test of anxiety. The testing apparatus is shaped like a '+' with two open arms perpendicular to two closed arms of equal dimensions. The closed arms are enclosed by three 10-cm high walls. Because rodents have an innate fear of elevated open spaces, they tend to spend less time in the open arms. Time spent in the open versus closed arms is taken as a measure of anxiety-like behaviour. Generally, treatment of rodents with anxiolytic drugs that reduce anxiety increases both the amount of time spent in and the number of entries into the open arms (Pellow et al., 1985). Test sessions lasted 5 min and the number of entries into the open arms and time spent in the open versus closed arms were recorded using an automated video-based tracking system (Noldus EthoVision 9, Netherlands).

2.5.3. Tail-suspension test (TST) of depression

The TST was first developed as a rodent screening test for potential human antidepressant drugs, and was performed as previously described (Cryan et al., 2005). Briefly, mice were suspended by their tails with adhesive tape attached to a suspension bar. The test sessions were recorded by a video camera and each session lasted 6 min. Immobility scores for each mouse were determined by manual scoring. The experimenters scoring the videos were blinded to the treatment and genotype. Reduced mobility is considered a measure of depressive-like behaviour.

2.5.4. Porsolt forced swim test (FST) of depression

The Porsolt FST was performed as described previously (Pouladi et al., 2009). Briefly, mice were placed in individual cylinders (25 cm tall × 19 cm wide) filled with room temperature water (23–25 °C) to a depth of 15 cm for a period of 6 min. The test sessions were recorded by a video camera placed directly above the cylinders. The sessions were examined blinded and the last 4 min of the test session was scored using a time-sampling technique to rate the predominant behaviour over 5-s intervals. The following behaviours were measured and recorded at the end of every 5 s: swimming/climbing and immobility.

2.6. Statistical analysis

Data are expressed as means ± SEM. Statistical significance was determined by one- or two-way ANOVA with appropriate *post hoc* testing, or by Student's t test. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Effect of clorgyline on brain MAO-A enzymatic activity and striatal neurotransmitter levels in wild-type mice

We first assessed the effect of different doses of clorgyline treatment (0.5, 1.5, or 3 mg/kg) on the enzymatic activity of MAO-A in the cortex of WT mice. Clorgyline treatment for 21 days resulted in a significant inhibition of MAO-A enzymatic activity compared with vehicle-treated animals (Fig. 2A; one-way ANOVA with Tukey's *post hoc* analysis; $p < 0.0001$). All three doses tested resulted in a reduction of approximately 80% in enzymatic activity, and no significant differences were
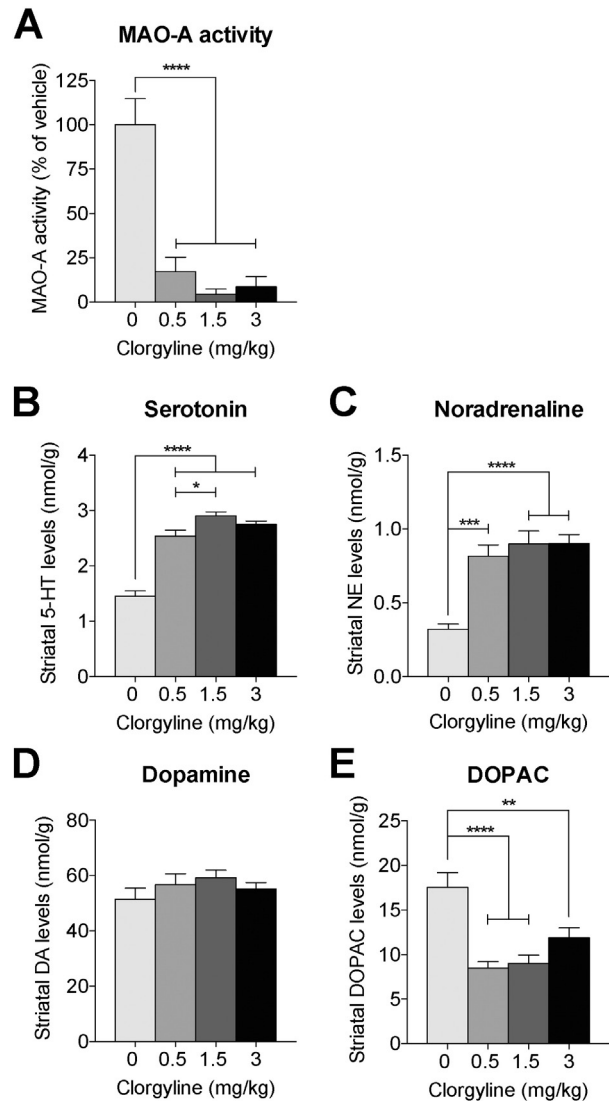
Fig. 2. Clorgyline treatment inhibits MAO-A enzymatic activity and increases striatal levels of monoamine neurotransmitters in WT mice. The three doses of clorgyline tested reduced approximately 80% of MAO-A enzymatic activity in cortical tissue from clorgyline-treated WT mice compared with vehicle-treated WT mice (A). MAO-A enzymatic inhibition resulted in significantly increased striatal levels of serotonin (B) and norepinephrine (C) in clorgyline-treated WT mice. Whereas levels of dopamine (D) were unchanged, striatal levels of DOPAC (E) were decreased after MAO-A enzymatic inhibition in clorgyline-treated WT mice. No clorgyline dose-effect was observed in any of the measures (A–E). (A) Values shown as percentage of vehicle-treated WT. Error bars represent the standard error of the mean; $n = 8–10$ mice/treatment group; ****$p < 0.0001$ by one-way ANOVA with Tukey's *post-hoc* test analysis. (B–E) Values shown as mean. Error bars represent the standard error of the mean; $n = 8–10$ mice/ treatment group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ by one-way ANOVA with Tukey's *post-hoc* test analysis.

observed between the three doses (Fig. 2A; one-way ANOVA with Tukey's *post hoc* analysis; n.s.).

To examine the effect of MAO-A inhibition on the metabolism of monoamine neurotransmitters, we assessed the levels of 5-HT, NE, and DA, as well as the levels of 3,4-Dihydroxyphenylacetic acid (DOPAC), the degradation product of dopamine, in the striatum of vehicle-treated WT mice. Treatment with clorgyline at all tested doses significantly elevated striatal levels of 5-HT and NE compared with vehicle-treated animals (Fig. 2B,C; one-way ANOVA with Tukey's *post hoc* analysis; $p < 0.0001$ for 5-HT; $p < 0.001$ and $p < 0.0001$ for NE). Interestingly, DA levels remained unchanged (Fig. 2D), whereas DOPAC levels were significantly decreased in clorgyline-treated WT mice,

suggesting that inhibition of MAO-A activity decreases the metabolism of DA (Fig. 2E; one-way ANOVA with Tukey's *post hoc* analysis; p < 0.001 and p < 0.0001).

3.2. Effect of clorgyline in tests of affective function

Having assessed the effect of different doses of clorgyline on the levels of MAO-A activity and its monoamine neurotransmitter substrates, we sought to evaluate the effect of clorgyline treatment on affective function in WT mice.

First, we examined possible detrimental effects of clorgyline on WT mice by measuring the body weight of all mice at the end of the 21 days of treatment. No significant difference in body weight was observed in mice treated with the low (0.5 mg/kg) and intermediate (1.5 mg/kg) doses of clorgyline (one-way ANOVA with Tukey's *post hoc* analysis; n.s.). However, treatment with the high dose (3 mg/kg)
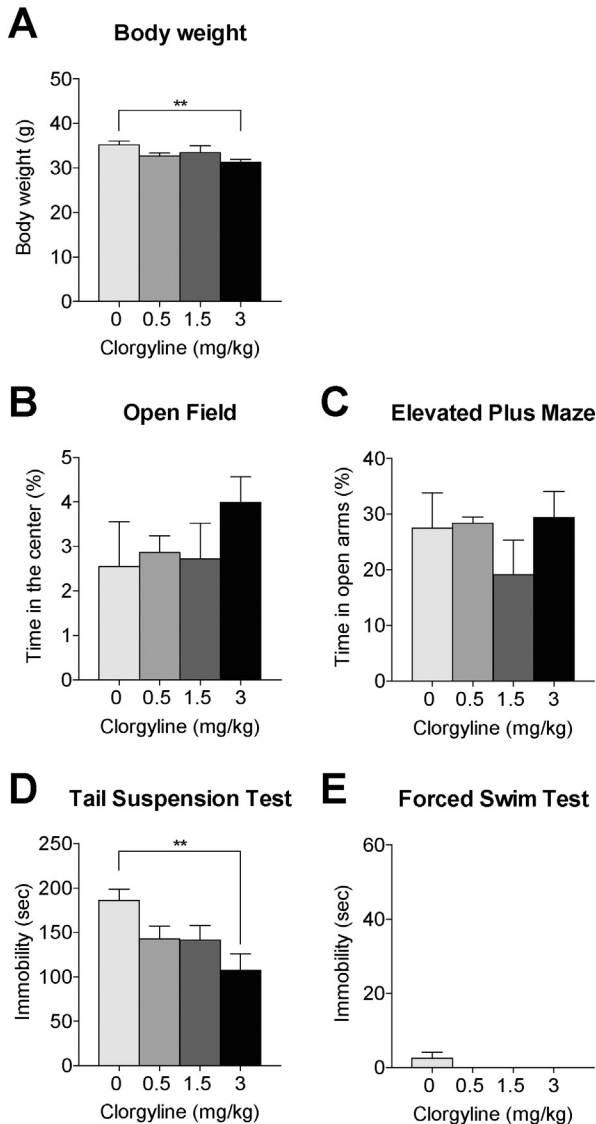
resulted in a significant loss of body weight when compared with vehicle-treated mice (Fig. 3A; one-way ANOVA with Tukey's *post hoc* analysis; p < 0.05), suggesting a potentially detrimental effect of the highest dose of clorgyline on WT mice.

We then assessed the effect of clorgyline on mouse anxiety levels using the open field (OF) and elevated plus maze (EPM) tests of anxiety-like behaviour. Clorgyline treatment had no effect on performance in these tests; time spent in the centre of the arena of the OF and in the open arms of the EPM were similar between the clorgyline- and vehicle-treated mice (Fig. 3B, C).

Next, we assessed depressive-like phenotypes using the tail suspension test (TST) and Porsolt forced swim test (FST). In the TST, mice treated with the high dose of clorgyline showed a significant decrease in depressive-like behaviour (immobility) compared with vehicle-treated mice (Fig. 3D). Clorgyline had no effect on performance in the FST at any of the doses tested (Fig. 3E).

These data indicate that clorgyline did not have a detrimental effect on anxiety- and depressive-like behaviour at any of the doses tested (Fig. 3B-E). However, because the highest dose of clorgyline (3 mg/kg) resulted in body weight loss, we chose the intermediate dose (1.5 mg/kg), the highest dose tested that did not cause detrimental effects, to evaluate the effect of clorgyline in the YAC128 mouse model of HD. To ensure similar pharmacokinetics of clorgyline in WT and YAC128 mice, we measured plasma levels of clorgyline following treatment and found no difference between the genotypes (data not shown).

3.3. Clorgyline treatment inhibits MAO-A activity and rescues striatal neurotransmitter deficits in YAC128 HD mice

Clorgyline treatment (1.5 mg/kg for 21 days) inhibited MAO-A enzymatic activity in cortical tissue from YAC128 HD mice by approximately
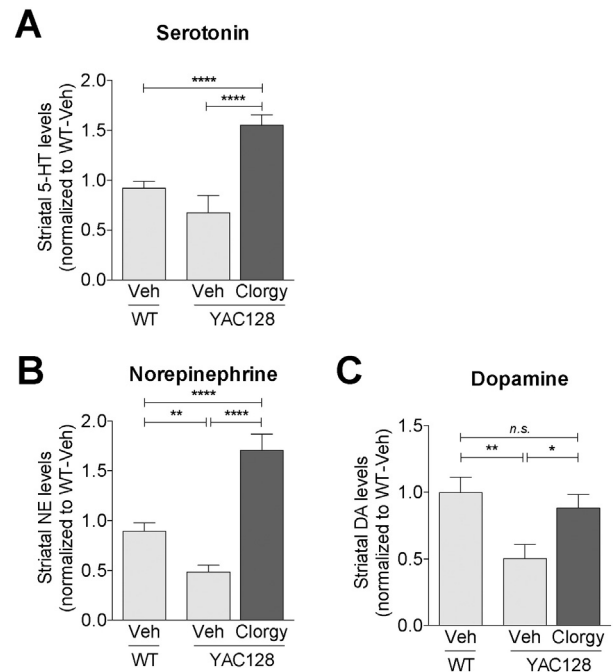
Fig. 3. Effect of clorgyline in tests of affective function. The clorgyline dose of 3 mg/kg decreased body weight in WT mice whereas no effect was observed in the other doses of clorgyline tested (A). In tests of anxiety-like behaviour, no effect of clorgyline was observed in WT mice in the open-field (B) and elevated plus maze (C) tests at any of the doses tested. In tests of depressive like-behaviour, WT mice treated with the highest dose of clorgyline (3 mg/kg) spent less time immobile in the tail suspension test (D), but no effect of clorgyline was observed in the forced swim test (E). (A–E) Values shown as mean. Error bars represent the standard error of the mean; n = 8–10 mice/treatment group; **p < 0.01 by one-way ANOVA with Tukey's *post-hoc* test analysis.

Fig. 4. MAO-A enzymatic inhibition by clorgyline rescues striatal neurotransmitter deficits in YAC128 HD mice. Striatal serotonin levels (A) were decreased in vehicle-treated YAC128 HD mice compared to vehicle-treated WT mice, whereas norepinephrine (B) and dopamine (C) striatal levels were significantly lower. Clorgyline treatment significantly increased serotonin (A) levels and rescued norepinephrine (B) and dopamine (C) levels in the striatum of clorgyline-treated YAC128 HD mice. (A–C) Values shown were normalized to vehicle-treated WT. Error bars represent the standard error of the mean; n = 18–21 vehicle-treated WT, n = 12–15 vehicle-treated YAC128, n = 18 clorgyline-treated YAC128 (three independent cohorts); *p < 0.05, **p < 0.01, ****p < 0.0001 by paired two-tailed student *t*-test.

90% (vehicle = 100 ± 26%, clorgyline = 12.3 ± 17.4%; paired two-tailed student t-test; p = 8.98×10^{-5}). Striatal DA and NE levels were also significantly reduced in YAC128 HD mice compared with WT mice (Fig. 4B,C; paired two-tailed student t-test; p = 0.004 for DA and p = 0.0018 for NE) however striatal 5-HT was not significantly altered in YAC128 HD mice compared with WT mice (Fig. 4A; paired two-tailed student t-test; ns). In clorgyline-treated YAC128 HD mice, striatal levels of all three neurotransmitters were significantly elevated in comparison with vehicle-treated YAC128 HD mice (Fig. 4; paired two-tailed student t-test; p = 7×10^{-4} for 5-HT; p = 3×10^{-6} for NE; p = 0.0158 for DA). Striatal 5-HT and NE levels were significantly elevated in treated YAC128 HD mice compared to vehicle-treated WT mice (Fig. 4A, B; paired two-tailed student t-test; p = 2×10^{-5} for 5-HT; p = 8×10^{-5} for NE; p = 0.45 for DA). To test the specificity of MAO-A inhibition by clorgyline, striatal levels of PEA, a specific MAO-B substrate, were also measured. No differences in striatal PEA were observed between vehicle- and clorgyline-treated YAC128 HD mice, suggesting lack of MAO-B inhibition (data not shown).

3.4. Clorgyline treatment improves affective function in YAC128 mice

Mouse models of HD, including the YAC128 HD model, show affective phenotypes, such as anxiety and depression (Pouladi et al., 2013, 2009, 2012b). Given the well-established relationship between changes in monoamine neurotransmitter levels and affective phenotypes, we evaluated the effect of clorgyline on behavioural measures of depression and anxiety in YAC128 HD mice. We found no difference in the body weight of clorgyline- and vehicle-treated YAC128 HD mice at the end of the 21-day treatment (Fig. 5A; paired two-tailed student t-test; p = 0.523), suggesting no detrimental effects of clorgyline treatment.

YAC128 HD mice showed an increased anxiety-like phenotype, signified by shorter time periods spent in the centre of the arena in the OF test and in the open arms of the EPM, compared with WT mice (Fig. 5B,C; paired two-tailed student t-test; p = 3×10^{-4} for OF and p = 2×10^{-5} for EPM). YAC128 HD mice also displayed depressive-like behaviour in the Porsolt FST, as shown by the increased immobility observed compared with WT mice (Fig. 5E; paired two-tailed student t-test; p = 6×10^{-4}). Performance in the TST was unaltered in HD mice compared with WT (Fig. 5D; paired two-tailed student t-test; p = 0.731).

Clorgyline treatment improved anxiety-like phenotypes, significantly increasing the amount of time spent in the centre of the arena in the OF test and in the open arms of the EPM (Fig 5B,C; paired two-tailed student t-test; p = 0.0063 for OF, p = 0.0315 for EPM). Decreased immobility times were also observed in the TST, but not the FST, indicating a reduction in depressive-like behaviour (Fig. 5D,E; paired student t-test; p = 0.029 for TST).

4. Discussion

Psychiatric manifestations are a common feature of HD (Rosenblatt, 2007). While social and psychological factors are thought to play a role (Paulsen et al., 2005), findings from human and animal studies strongly implicate neurobiological alterations related to the HD mutation in the aetiology of psychiatric disturbances of HD (Pouladi et al., 2009). Although the aetiology of depression in HD remains poorly understood, several pathogenic mechanisms have been proposed, including deficient brain-derived neurotrophic factor (BDNF) and neurotrophin signalling, a hyperactive hypothalamic–pituitary–adrenal (HPA) axis, and impaired hippocampal neurogenesis (Ben M'Barek et al., 2013; Du et al., 2013). In addition to these abnormalities (Pouladi et al., 2012a; Simpson et al., 2011), we now demonstrate that YAC128 HD mice exhibit deficits in monoamine neurotransmitters known to be tightly associated with affective disorders (Hamon and Blier, 2013; Lanni et al., 2009). We further show that elevating the levels of these monoamines,
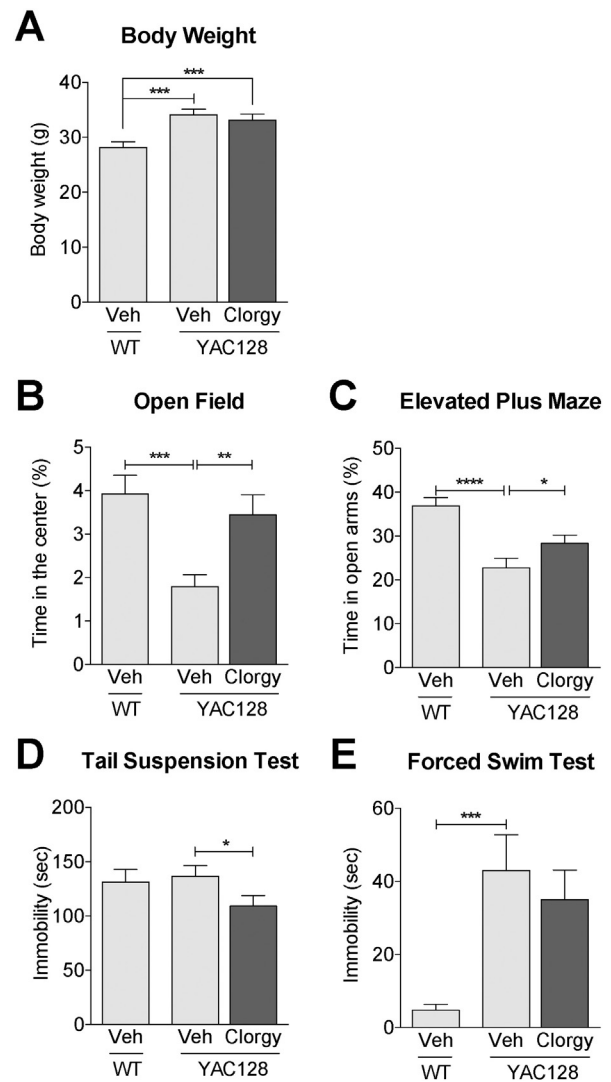
Fig. 5. Clorgyline treatment improves affective function in YAC128 HD mice. Clorgyline-treated YAC128 HD mice did not show changes in body weight compared with YAC128-vehicle HD mice (A). In tests of anxiety-like behaviour, YAC128-vehicle mice presented anxiety-like phenotypes in the open field (B) and elevated plus maze (C) tests compared with WT-vehicle mice. Clorgyline treatment ameliorated the anxiety-like deficits observed in the YAC128 HD mice (B-C). In tests of depressive-like behaviour, vehicle-treated YAC128 HD mice exhibited depressive-like phenotypes in the Porsolt forced swim test (E) but not in the tail suspension test (D) compared with vehicle-treated WT mice. Clorgyline treatment reduced the time that YAC128 HD mice spent immobile in the tail suspension test (D), but no effect was observed in the forced swim test (E). (A–E) Values shown were normalized to vehicle-treated WT mice. Error bars represent the standard error of the mean; n = 19–21 vehicle-treated WT, n = 17–20 vehicle-treated YAC128, n = 21–22 clorgyline-treated YAC128 (three independent cohorts); *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by paired two-tailed student t-test.

namely serotonin, norepinephrine, and dopamine, by pharmacological inhibition of MAO-A improves affective phenotypes of YAC128 HD mice. These findings suggest that monoaminergic impairments may be a contributing factor to the psychiatric manifestations of HD.

Deficits in dopaminergic signalling have been demonstrated in presymptomatic gene carriers, symptomatic patients, and animal models of HD (Callahan and Abercrombie, 2011; Cha et al., 1999; Chen et al., 2013; Mochel et al., 2011; Pavese et al., 2003, 2010; Renoir et al., 2014). Similarly, there is evidence for serotonergic dysfunction in rodent models and in patients with HD (Jahanshahi et al., 2013; Manyam, 1973; Mochel et al., 2011; Pang et al., 2008; Renoir et al., 2011; Rosas et al., 2015). Treatments targeting the dopamine-norepinephrine (Renoir et al., 2012) and the serotonin systems (Pang et al., 2008) have been shown to improve

affective function in rodent models of HD, indicating that these deficits contribute to affective abnormalities. Interestingly, improvements in affective phenotypes resulting from targeting the serotonergic system have been observed in rodents expressing an N-terminus fragment of mutant HTT (Pang et al., 2008) but not those expressing the full length HTT protein (Hult Lundh et al., 2013; Pouladi et al., 2009). Serotonergic deficits may therefore be more important in the development of affective phenotypes in HTT N-terminus fragment models, which generally have a more rapid onset.

In addition to our findings with clorgyline, an MAO-A inhibitor, treatments with the mood stabilizers, lithium and valproate, have also been reported to improve affective phenotypes in YAC128 HD mice (Chiu et al., 2011). Indeed, valproate treatment improved performance in the FST and TST tests of depression, and combined valproate and lithium treatment improved performance in the FST, TST, as well as the open-field test of anxiety (Chiu et al., 2011). These improvements were accompanied by altered GSK-3beta activation, histone H3 acetylation, and improved expression of HSP70, BDNF, and its cognate receptor TrkB, which may have contributed to the observed improvements (Chiu et al., 2011). Thus, multiple pathogenic mechanisms may underlie affective dysfunction in HD. While it would be tempting to speculate about the relative contribution of these pathways and hence the therapeutic value of engaging each in isolation on affective phenotypes in HD, a combinatorial treatment strategy is likely to yield greater benefit, and determining the optimal combination of therapeutic agents should be the subject of future studies.

It should be noted that at the dose chosen in this study, clorgyline treatment elevated serotonin and norepinepherine in YAC128 HD mice to levels higher than those seen in WT mice. This observation highlights the need for appropriate dose titration to ensure that neurotransmitters affected in HD are restored without overtly exceeding the normal range and risking undesirable side effects such as the serotonin syndrome (Iqbal et al., 2012).

Several factors may contribute to the deficits in the levels of striatal monoamine neurotransmitters observed in YAC128 HD mice. The rescue of neurotransmitter levels we observed following clorgyline treatment supports a role for MAO-A. Dysregulation of MAO-A/B activity has been linked not only to depression but also to neurodegenerative disorders. Indeed, increased MAO-A/B activity has been observed in patients with HD in brain regions that undergo neurodegeneration (Richards et al., 2011) and in human neural cells differentiated from HD patient-derived induced pluripotent stem cells (Ooi et al., 2015). It is plausible that monoamine deficits reflect altered biosynthesis, although this requires further investigation.

There is considerable variability in the nature, severity, and timing of appearance of psychiatric symptoms in HD (Rosenblatt, 2007; van Duijn et al., 2007). While this variability may, in part, reflect poor standardisation of the diagnostic criteria used to assess psychiatric features, it also points to a likely role for environmental (Nithianantharajah and Hannan, 2006), epigenetic (Glajch and Sadri-Vakili, 2015), and genetic modifiers (Consortium et al., 2015). Genetic polymorphisms and epigenetic variation in *MAOA* have been associated with altered MAO-A levels and activity (Naoi et al., 2015; Sabol et al., 1998; Shumay et al., 2012) as well as psychiatric disorders, including depression, aggression, and anxiety (Buckholtz and Meyer-Lindenberg, 2008; Naoi et al., 2015). Furthermore, MAO-A expression has been shown to be responsive to certain environmental factors (Naoi et al., 2015). Thus both genetic and epigenetic variation in *MAOA* may contribute to psychiatric manifestations in HD.

The results from our study support a role for monoaminergic impairments in the affective phenotypes observed in YAC128 HD mice, and suggest potential therapeutic benefits of MAO-A inhibitors for the treatment of psychiatric abnormalities in HD. Our findings also raise the question of whether targeting monoaminergic impairments in HD may improve symptoms beyond psychiatric abnormalities, including deficits in motor function and cognition.

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