Multiple cholinesterase inhibitors have antidepressant-like properties in the mouse forced swim test

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**ABSTRACT**

There is high clinical interest in improving the pharmacological treatment of individuals with Major Depressive Disorder (MDD). This neuropsychiatric disorder continues to cause significant morbidity and mortality worldwide, where existing pharmaceutical treatments such as selective serotonin reuptake inhibitors often have limited efficacy. In a recent publication, we demonstrated an antidepressant-like role for the acetylcholinesterase inhibitor (AChEI) donepezil in the C57BL/6J mouse forced swim test (FST). Those data added to a limited literature in rodents and human subjects which suggests AChEIs have antidepressant properties, but added the novel finding that donepezil only showed antidepressant-like properties at lower doses (0.02, 0.2 mg/kg). At a high dose (2.0 mg/kg), donepezil tended to promote depression-like behavior, suggesting a u-shaped dose-response curve for FST immobility. Here we investigate the effects of three other AChEIs with varying molecular structures: galantamine, physostigmine, and rivastigmine, to test whether they also exhibit antidepressant-like effects in the FST. We find that these drugs do exhibit therapeutic-like effects at low but not high doses, albeit at lower doses for physostigmine. Further, we find that their antidepressant-like effects are not mediated by generalized hyperactivity in the novel open field test, and are also not accompanied by anxiolytic-like properties.

These data further support the hypothesis that acetylcholine has a u-shaped dose-response relationship with immobility in the C57BL/6J mouse FST, and provide a rationale for more thoroughly investigating whether reversible AChEIs as a class can be repurposed for the treatment of MDD in human subjects.

1. Introduction

Major Depressive Disorder (MDD) is a frequently occurring neuro-psychiatric disorder throughout the world, and remains a prevalent source of morbidity and mortality [1–3]. Existing pharmaceutical treatments for MDD, such as selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs), typically act on the monoaminergic signaling pathways that include serotonin, norepinephrine, and dopamine. While these drugs can effectively treat MDD in some individuals, others do not respond well to them and may also encounter significant side effects while taking them, creating a need for new types of pharmacological treatments for MDD [4,5].

In a recent publication [6], our research group demonstrated that the AChEI donepezil has antidepressant-like properties in male C57BL/6J mice in the FST. Although a limited number of preclinical studies [7–9] had found similar effects with AChEIs, those results and ours are perhaps surprising because acetylcholine has historically been more associated with cognition than mood [10,11], where deficits may result in cognitive impairment in Alzheimer’s disease [12,13]. When acetylcholine, and by extension cholinesterase inhibition, has been implicated in mood regulation, it has mostly been thought to promote depression-like behavior in rodents [14–19], consistent with the cholinergic-adrenergic hypothesis of mania and depression [20,21]. The implication of acetylcholine in mood regulation may not be unexpected, however, because this neurotransmitter interacts with the monoamines through an overlapping spatial distribution in the brain, including prefrontal cortex [22–24].

In our previous publication [6], we demonstrated that donepezil shows a u-shaped dose-response curve for immobility in the male C57BL/6J mouse FST, in which lower doses (0.02, 0.2 mg/kg, i.p.) can produce an antidepressant-like decrease in immobility (or increase in swimming or climbing), and a higher dose (2.0 mg/kg) tended to increase immobility. We also showed that the antidepressant-like properties of the two lower doses of donepezil are not associated with...
generalized hyperactivity in the novel open field test (OFT), while also not having anxiolytic-like effects. The higher dose, in contrast, suppressed locomotion in the OFT, while also possibly having an anxiogenic-like effect.

In the current study, we aim to investigate whether the therapeutic-like effects of donepezil in the FST generalize to other reversible AChEIs. We studied three commonly used and clinically available drugs in this class in male mice: galantamine, physostigmine, and rivastigmine. The depression-related properties of physostigmine, in particular, have been investigated more extensively in rodents than the other two drugs, and we sought comparison with this literature. Further, if all four drugs have antidepressant-like effects in the FST, this would suggest that the class of reversible AChEIs in general has mood-related therapeutic-like properties in rodents. To further test how general these drug effects are, we also investigated the therapeutic-like effects of donepezil in female mice. We used donepezil in these additional female experiments because it is the AChEI for which we have the most evidence of antidepressant-like effects.

2. Methods

2.1. Subjects

One hundred ninety-two experimentally naïve adult (8–9 weeks old upon arrival) male C57BL/6J mice and 64 adult females (256 total mice) were obtained from a commercial supplier (The Jackson Laboratory, Bar Harbor, ME). In particular, Experiments 1–6 each used a separate cohort of 32 male mice (four groups of n = 8 per drug dose), and Experiments 7–8 each used a separate cohort of 32 females. We used this moderate sample size (n = 8) because mouse FST experiments of antidepressants such as fluoxetine, imipramine, or bupropion, typically have reported relatively large effect sizes (>1.0) and often used this approximate sample size per group [25–27]. To assign animals to drug groups, we used a random number generator. No blinding of the experimenter to the drug groups was carried out because we used an automated behavioral scoring procedure (see below). Upon arrival and throughout the experiments, mice were group housed in cages within a humidity- and temperature-controlled vivarium, and kept on a 12:12 h light/dark cycle (lights on at 6 a.m.) with ad libitum access to food and water. Each cage had an Enviropak (Lab Supply, Fort Worth, TX) for enrichment and use in nest building. All mice were handled daily by the experimenter (for approximately 30 s per day) for the first five days upon arrival, to acclimate them to the experimenter (PJF). The experimenter also tail-marked the mice upon arrival, and re-tailed marked them every three days throughout the experiments. All experiments were carried out in the daytime during the light phase. The first behavioral test of each experiment (Day 0) was carried out one to two weeks after the mice arrived in our facility. All procedures were conducted at the University of Michigan and were performed in strict accordance with the guidelines and regulations set forth by the National Institutes of Health and the University of Michigan, with full approval from its Institutional Animal Care and Use Committee (Protocol number: PRO00007803). The eight experiments described here, while related to one another and having some degree of overlapping information, were not repeated.

2.2. Drugs

Rivastigmine (tartrate) and galantamine (Cayman Chemical, Ann Arbor, MI), as well as physostigmine (salicylate) (Sigma-Aldrich, St. Louis, MO), were dissolved in a 0.9 % saline vehicle solution. As in our previous study [6], donepezil was prepared in a vehicle solution consisting of five percent Tween 20 (Sigma-Aldrich, St. Louis, MO) dissolved in saline (0.9 %) (v/v). All mice were injected intraperitoneally (i.p.) at a volume of 10 mL/kg, with drug solutions, 30 min prior to behavioral testing. We chose 30 min since it is a widely used time delay to study the acute effects of monoaminergic antidepressants in behavioral assays such as the FST and open field test, and we wanted our data on AChEIs to be directly comparable with that literature. We also used this delay in our previous donepezil study [6]. Galantamine and rivastigmine were administered in the same doses that we had previously used for donepezil (0.02, 0.2, 2.0 mg/kg). We also began our experiments with physostigmine using 0.02, 0.2, and 2.0 mg/kg, but quickly found that the highest dose was toxic, and three mice had to be excluded from Experiment 3 because of this. So we scaled the doses of physostigmine to be 10-fold lower, and this produced a u-shaped dose-response curve, similar to the (higher) dosage ranges we used for the other three drugs.

2.3. Forced swim and open field tests

For a detailed description of these tests, see our previous paper [6]. Briefly, for the FST, two mice were tested simultaneously in a pair of clear Plexiglas cylinders, 30 cm tall and 20 cm in diameter, filled halfway with water that was 24 ± 1 degree C. Mice were acclimated to the behavior room (illuminated to 300 lx throughout acclimation and testing) for approximately one hour before testing began. Each FST trial lasted six minutes, but behavior was only scored during the last four minutes. Behavior was quantified with a horizontally mounted camera, and a software package (EthoVision XT, Noldus Information Technology, Leesses, VA) was used for subsequent analysis. At the end of each behavioral trial, the mouse was removed from the tank, dried with a paper towel, and returned to its homecage.

For the OFT, two mice were tested simultaneously in adjacent open field boxes that had opaque white Plexiglas walls (40 × 40 × 40 cm). Each box was positioned on the floor of the room and illuminated with indirect white lighting to approximately 40 lx in the corners and 80 lx in the center. At the beginning of each trial (10 min duration), the mouse was placed in a corner of the box, facing the center. A vertically mounted camera recorded the position and movement of the mouse, for subsequent analysis in EthoVision XT, including whether the animal was in the unmarked center square (20 × 20 cm). When each trial ended, the mouse was removed from the box and returned to its homecage. The box was cleaned with 70 % ethanol between trials.

2.4. Analysis and statistics

For a detailed description of analysis methods, see our previous paper [6]. We initially analyzed data with parametric statistics (GraphPad Prism, GraphPad Software, La Jolla, CA). Mice that exhibited outlier behavior > 2 standard deviations from the mean (of the cohort for each drug dose) for a given FST or OFT behavior were removed from that analysis (see Table S1 for raw forced swim data for each mouse, and Table S2 for number of animals excluded from each experiment). One-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparisons test were used to determine if a given AChEI drug modulated FST or OFT behavior, and if it did whether the drug groups differed significantly from the vehicle group for that test. Results are shown as mean ± standard error of the mean (SEM). Variance was similar between the groups that are being statistically compared. We also performed a randomization analysis to further investigate whether these drugs produced a u-shaped, or inverted u-shaped, dose-response curve for climbing, swimming, and immobile behavior in the FST (see Fitzgerald et al. (2020) for details).

3. Results

In the eight experiments (Expts 1–8; a separate group of 32 animals each) that comprised this study, we carried out the mouse FST to investigate antidepressant-related properties of galantamine, physostigmine, rivastigmine (and donepezil in females) as well as the open field test (OFT) in Expts 2, 4, 6, and 8. As with donepezil previously, we found that these three drugs did not exhibit robust antidepressant-like effects during the first forced swim session in behaviorally naïve
animals (Expts 1, 3, 5), so we carried out at least four FSTs in each of these groups of mice, each separated by one or two weeks to allow almost all of the drug to be eliminated physiologically between tests.

We also repeatedly “crossed over” the drug groups (as illustrated in Figure S1), such that animals previously given vehicle injections were next given 0.02 mg/kg galantamine (Expts 1, 2), and vice versa, where we also swapped 0.2 mg/kg with 2.0 mg/kg. We did the same with rivastigmine (Expts 5, 6). For physostigmine (Expts 3, 4), we swapped 0.002 mg/kg and vehicle, and also swapped 0.02 and 0.2 mg/kg. We performed these crossovers to further investigate whether the same group of mice would respond differently to another dose of these drugs. In Expts 2, 4, and 6, we first carried out the OPT twice to investigate whether each drug’s putative antidepressant-like properties are confounded by generalized hyperactivity, and also to test whether these drugs modulate anxiety-related behavior. After these two OFTs, we carried out two FSTs in each cohort to compare forced swim with open field behavior in the same animals. Thus our overall experimental design was similar to that used in our donepezil study [6].

3.1. Experiment 1

In the first experiment (Fig. 1), we studied the AChEI galantamine in six FSTs. In FST1, climbing was not significantly modulated by drug, but swimming (one-way ANOVA: \(F(3,27) = 4.48; p < 0.05\)) and immobile (\(F(3,27) = 3.52; p < 0.05\)) were, driven by depression-like effects at the high dose (Dunnett’s multiple comparisons test, each adjusted \(p < 0.05\)).
It should also be noted that the three drug groups formed an “inverted v” (climbing, swimming) or “upright v” (immobile) that qualitatively resembles FST1 with donepezil (Fig. 2, Fitzgerald et al. (2020)) and FST1 with rivastigmine from the current study (Figure S2). These two terms are used to describe the overall shape of the three bars in the graphs representing the three doses of galantamine (and not including the fourth bar representing vehicle). The inverted v for climbing and swimming in Fig. 1, FST1 describes the local maximum for the medium dose (0.2 mg/kg, orange bar), flanked by the two lower values for the low (yellow bar) and high (red bar) doses, thereby forming an inverted v shape. In contrast, these three bars for immobility form an upright v, where the medium dose is a local minimum.

In the crossed-over FST2, climbing showed a trend toward modulation by drug ($p = 0.063$), and swimming showed significant modulation ($F(3,28) = 3.22; p < 0.05$), driven by a depression-like effect at the high dose ($p < 0.05$). Immobility was not significantly modulated.

FST3, crossed back over to match FST1, qualitatively resembled FST1 and its inverted or upright v’s, with a tendency toward depression-like effects at the low and high doses.

FST4, crossed back to match FST2, did not show significant modulation of climbing, although this measure was qualitatively inverted u-shaped, similar to swimming ($F(3,28) = 4.50; p < 0.05$). Swimming was strongly inverted u-shaped in the randomization test ($p < 0.01$). Immobility was not significantly modulated in the one-way ANOVA, but the randomization test found that it was u-shaped ($p < 0.05$).

Since at this point we had observed greater antidepressant-like tendencies in FST2 and 4, relative to FST1 and 3, suggesting an alternating pattern, we next kept the drug groups the same as in FST4 and ran FST5 14 days later, to see if this would disrupt the alternations. FST5 showed robust antidepressant-like effects of galantamine. Climbing ($F(3,27) = 5.50; p < 0.01$), swimming ($F(3,28) = 7.55; p < 0.01$), and immobile ($F(3,27) = 7.36; p < 0.01$) all were significantly modulated, with the medium dose being antidepressant-like relative to vehicle in all cases (each adjusted $p < 0.05$). All three swim behaviors were also modulated by drug in the randomization test (each $p < 0.01$).

FST6, crossed back to match FST1 and FST3, did not show significant modulation by drug, but was slightly qualitatively depression-like at the low dose.

In summary for this experiment, antidepressant-like properties for low doses of galantamine tended to emerge during FST4 and FST5.

3.2. Experiment 2

In the second experiment (Fig. 2), we investigated the effects of galantamine in two OFTs and subsequently two FSTs.

In OFT1, total distance, center entries, and percent center time were all modulated by drug (each one-way ANOVA $p < 0.01$), with the high dose suppressing exploration or percent center time relative to vehicle for all three measures (each adjusted $p < 0.01$).

OFT2, where the drug groups were crossed over, also showed suppression of exploration at the high dose for total distance (adjusted $p < 0.01$), center entries ($p < 0.05$), and percent center time ($p < 0.093$).

FST1, crossed back to match OFT1, did not show significant modulation of climbing, swimming, or immobile by galantamine (each one-way ANOVA $p > 0.05$). FST2 also did not reveal antidepressant-like effects, with the high dose either trending toward being depression-like...
like (climbing: adjusted $p = 0.054$; swimming: adjusted $p = 0.081$) or reaching significance (immobile: adjusted $p < 0.05$).

### 3.3. Experiment 3

In the first swim session (FST1) of the third experiment (Fig. 3), physostigmine treatment did not significantly modulate climbing, swimming, or immobility, as analyzed with one-way ANOVAs (each $p > 0.05$). Climbing did however exhibit a qualitatively mild, inverted u-shape.

For FST2, carried out seven days later, we crossed over the drug groups to see if this would produce an antidepressant-like effect. One-way ANOVAs again did not reveal significant modulation by drug, although the data showed mild inverted u-shapes (climbing, swimming). We correspondingly saw an upright u-shape for immobile.

In FST3, 14 days later, we crossed back to the FST1 groupings and again did not observe an antidepressant-like effect.

FST4, crossed back to match FST2, did not show significant drug modulation for climbing or immobile, although the latter was qualitatively u-shaped. Swimming, however, was significantly modulated by physostigmine (one-way ANOVA: $F(3,24) = 3.24; p < 0.05$), driven by a statistical trend toward an increase at the low dose relative to vehicle (adjusted $p = 0.097$). Our randomization test confirmed this inverted u-shape for swimming ($p < 0.05$).

In FST5, which matched the drug groups from FST4, climbing was significantly modulated by drug dose (one-way ANOVA: $F(3,24) = 5.84; p < 0.01$), with the strongest difference from vehicle being an increase in climbing at the medium dose (adjusted $p = 0.11$). Swimming was not significantly modulated in the one-way ANOVA ($p = 0.12$) but qualitatively showed an inverted u-shaped curve. Immobile was also not significantly modulated in the one-way ANOVA ($p = 0.13$) but did exhibit a decrease at the medium dose.

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![Figure 3](image-url)

**Fig. 3.** Antidepressant-like effects of physostigmine also emerge after repeated, crossed-over FSTs. Shown are the six FSTs from Experiment 3, where a gap of seven or 14 days was used for shorter versus longer washout periods, respectively, between swims. After FST1, the vehicle and 0.002 mg/kg dose groups were switched (“crossed over”), as were the 0.02 and 0.2 mg/kg groups, for FST2. Such crossing over was also carried out in the subsequent FSTs, where maroon color indicates the original drug grouping, and green indicates the crossed over grouping, where FST1 = FST3 = FST6, and FST2 = FST4 = FST5. Error bars represent mean ± SEM. For bars: * adjusted $p < 0.05$ versus vehicle (or adjusted $p$ value is explicitly shown for a statistical trend). ** p < 0.05 for inverted u-shape.
For FST6, we crossed back over to the FST1 and FST3 groupings, and unlike in our earlier donepezil experiments [6], we observed antidepressant-like effects. Climbing was robustly modulated by physostigmine treatment (one-way ANOVA: F(3,24) = 4.47; p < 0.05) with the medium dose significantly higher than vehicle (adjusted p < 0.05), where this inverted u-shape was verified by the randomization test (p < 0.05). Swimming was not strongly modulated, but immobility showed a strong trend (one-way ANOVA: F(3,24) = 2.95; p = 0.053), with an additional trend toward a decrease at the medium dose (p = 0.088).

In summary, physostigmine exhibited some degree of a u-shaped dose-response curve for immobility (and inverted u-shape for climbing and swimming) especially in the later swims.

3.4. Experiment 4

In the fourth experiment (Fig. 4), we first carried out two OFTs with physostigmine, followed by two FSTs.

In OFT1, total distance traveled was modulated by drug (one-way ANOVA: F(3,28) = 12.95; p < 0.01), driven by a decrease at the high dose (adjusted p < 0.01). Center entries (F(3,28) = 5.55; p < 0.01) and percent center time (F(3,27) = 4.33; p < 0.05) showed a similar qualitative pattern to distance traveled.

We crossed the drug groups over for OFT2 and saw decreases in the three measures not only at the high dose (each adjusted p < 0.01), but also for the medium dose in center entries (p < 0.05), and for the low (p < 0.05) and medium (p < 0.01) doses in percent center time.

In FST1, two weeks later and crossed back to match OFT1, we only observed depression-like effects with physostigmine. For climbing, the medium and high doses were significantly depression-like relative to vehicle (each adjusted p < 0.01). This was also true in swimming for the high dose (p < 0.01). For immobile, the low (p < 0.05), medium (p < 0.05), and high (p < 0.01) doses were depression-like.

FST2, crossed back to match OFT2, produced results that were qualitatively different from FST1. While the one-way ANOVA calculations were not significant for any of the three measures (each p > 0.05), the low dose suggested a mild antidepressant-like effect for climbing (F(3,26) = 2.82; p = 0.059), as well as for immobile (F(3,27) = 2.40; p = 0.090).

3.5. Experiment 5

In the fifth experiment (Figure S2), we addressed whether another AChEI, rivastigmine, has antidepressant-like effects in the FST. In FST1, climbing behavior was modulated by drug (one-way ANOVA: F(3,27) = 3.35; p < 0.05), with the high dose being depression-like relative to vehicle (adjusted p < 0.05). Rivastigmine also affected swimming behavior (p < 0.05) and showed a trend toward altering immobility (p = 0.063). Collectively these results for FST1 revealed no antidepressant-like effects.

In FST2, we crossed over the drug groups and found that this drug modulated climbing (F(3,27) = 4.04; p < 0.05), driven by a mild increase in climbing for the low dose and decreases for the higher two doses. Swimming was also modulated (p < 0.01) and mildly inverted u-

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**Fig. 4.** Antidepressant-like effects of low dose physostigmine may not be due to generalized hyperactivity. Shown are the results from Experiment 4, consisting of a new cohort of mice that was given the open field test (OFT) twice, followed by two FSTs. A gap of seven or 14 days was used for shorter versus longer washout periods, respectively, between OFTs or FSTs. Crossing over of drug groups was the same as in Experiment 3 (maroon grouping: OFT1 = FST1; green grouping: OFT2 = FST2). Error bars represent mean ± SEM. For bars: * adjusted p < 0.05 versus vehicle, ** adjusted p < 0.01 versus vehicle (or adjusted p value is explicitly shown for a statistical trend).
shaped (confirmed by the randomization test; \( p < 0.05 \)), driven by a decrease at the high dose (adjusted \( p < 0.05 \)). Immobility qualitatively showed a u-shaped curve (F(3,28) = 4.67; \( p < 0.01 \)), with a mild decrease at the low dose and a statistical trend toward an increase at the high one (adjusted \( p = 0.058 \)). Thus there may have been mild antidepressant-like effects in FST2.

For FST3, we crossed back over to the FST1 groupings and again did not observe antidepressant-like effects. Climbing was not modulated by rivastigmine (\( p > 0.05 \)), whereas swimming showed a depression-like decrease at the high dose (adjusted \( p < 0.01 \)), and immobility had a depression-like increase at this dose (adjusted \( p < 0.05 \)).

In FST4, we crossed back to the FST2 groupings but only observed depression-like effects driven by the high dose, in climbing, swimming, and immobility (each adjusted \( p < 0.01 \)).

Thus in FST1–4, rivastigmine consistently showed depression-like effects at the high dose, while possibly exhibiting mild antidepressant-like properties at the low dose during FST2.

3.6. Experiment 6

In the sixth experiment (Figure S3), we ran two OFTs with rivastigmine, followed by two FSTs with this drug.

In OFT1, this drug (much like physostigmine and galantamine) produced decreases in exploratory behavior as well as percent center time at the high dose (each adjusted \( p < 0.01 \)), with a trend in this direction for the medium dose (adjusted \( p = 0.085 \)) in distanced traveled. In fact, there were zero center square entries by all of the eight high dose mice in OFT1, as well as OFT2.

OFT2, crossed over and run a week later, was qualitatively similar to OFT1, with suppression of all three measures at the high dose (each adjusted \( p < 0.01 \)), and the medium dose for percent center time (\( p < 0.01 \)).

FST1, crossed back to match OFT1, showed antidepressant-like inverted u-shaped distributions for climbing (F(3,23) = 3.00; \( p = 0.051 \)) and swimming (F(3,23) = 5.50; \( p < 0.01 \)). Conversely, immobile exhibited a u-shaped distribution (F(3,23) = 5.30; \( p < 0.01 \)), with a trend toward the medium dose being significantly lower than vehicle (\( p = 0.084 \)). The inverted u-shapes (climbing, swimming) and upright u-shape (immobile) were confirmed by the randomization test (each \( p < 0.01 \)). Five mice had to be excluded from the analysis of FST1 because they were prematurely removed from the tank due to trouble staying afloat.

FST2, crossed back to match OFT2, did not show modulation by rivastigmine for any of the three measures (each one-way ANOVA: \( p > 0.05 \)).

Experiments 7 and 8, in which we tested the effects of donepezil in the FST and OFT in female mice, are further described in the Supplementary Information.

4. Discussion

In this study, we have extended the findings of Fitzgerald et al. (2020) and shown that a range of reversible AChEIs have antidepressant-like properties in the C57BL/6J mouse FST. In particular, these drugs appear to have u-shaped dose-response properties with respect to immobility in the FST, with high doses having depression-like effects and low doses showing antidepressant-like effects. These therapeutic-like effects tend not to be present in the first swim session, and only emerge in later swims, and possibly are modulated by associative learning since they emerge most strongly when specific cohorts are re-exposed to therapeutic doses. Furthermore these effects involve increases in either climbing or swimming behavior, as well as decreases in immobility. Finally, the antidepressant-like properties of low doses are not accompanied by generalized hyperactivity or anxiolytic-like effects in the OPT. In the rest of the Discussion, we address these points and related ones in greater detail, framing them within the rodent and human subjects literature.

Our findings from the current study, in addition to those we presented on donepezil previously [6], add to a small but growing literature that suggests some AChEIs have antidepressant-like properties in rodent models. Papp et al. (2016) have previously shown in chronically stressed rats that donepezil and rivastigmine have antidepressant-like properties in the sucrose preference test, at least when these drugs are administered chronically [7]. A study of olfactory bulbectomized mice found that chronic administration of rivastigmine led to antidepressant-like effects in the FST, although the authors suggested that this drug was acting through the serotonergic 5HT1A receptor [9]. In a third study, Maurice et al. (2006) demonstrated that swiss mice exhibit decreased immobility in the FST when up to very high doses of donepezil are administered acutely, although they failed to find these effects with rivastigmine or another AChEI, tacrine [8]. Maurice et al. attributed the therapeutic effects of donepezil to a non-cholinergic mechanism: modulation of the sigma-1 receptor. We suggest here that in C57BL/6J mice (and possibly other strains), a broad range of reversible AChEIs (donepezil, galantamine, physostigmine, rivastigmine) have antidepressant-like properties in the FST, possibly through cholinergic mechanisms in all cases. Since these four drugs inhibit the acetylcholinesterase enzyme and have very different molecular structures, yet share antidepressant-like effects in the FST in our hands, it is likely that they are doing so through cholinergic mechanisms rather than another single off target receptor or signaling pathway that would be expected to interact differently with their divergent structures. One possibility is that donepezil has more robust antidepressant-like properties than galantamine, physostigmine, and rivastigmine, but uncertainty regarding the optimal doses for each drug (and timing of our experiments) precludes direct, definitive comparison between our various experiments. It is also interesting that while our results are isolated to the FST, others point to effects in sucrose preference [7] which is behaviorally quite different and likely demands different brain structures. This efficacy across a variety of behaviors and AChEI types suggests a possible broadly-impacting, cholinergically-based antidepressant-like effect. This topic should be studied in further detail.

While the neurochemical and circuit-based mechanisms through which low dose AChEIs achieve their antidepressant-like effects remains a topic for future investigation, we suggest here that they may do so by interacting with monoaminergic neurotransmitters and altering signaling in already established mood-related circuits. Acetylcholine is known to interact with serotonergic and noradrenergic signaling, partly because their receptors are in some instances localized to the same neurons [24]. This mood-related interaction with monoaminergic signaling could be achieved through either nicotinic or muscarinic cholinergic receptors, where nicotine itself is already known to have antidepressant-like properties in rodents [28,29]. Another possibility is that AChEIs achieve their putative antidepressant-like effects in a manner that is independent of monoaminergic signaling. In either case, cholinergic signaling is already known to modulate key mood-related circuits such as medial prefrontal cortex [30] and ventral hippocampus [31,32], which could play a prominent role in the behavioral effects we observed here.

Rodent microdialysis studies, carried out with AChEIs such as donepezil and physostigmine, provide strong evidence that these drugs boost the extracellular concentration of acetylcholine in diverse circuits such as medial prefrontal cortex and hippocampus [33,34]. Physostigmine is particularly potent at boosting this neurotransmitter [33], consistent with our data here which suggest greater potency of this drug relative to the other three AChEIs we have tested. AChEIs have also been shown to modulate the extracellular concentrations of dopamine, serotonin, and norepinephrine in a variety of circuits, suggesting interaction with cholinergic signaling [34,35].

The suggestion that a range of reversible AChEIs have antidepressant-like effects in the rodent FST, may modify but not necessarily contradict the cholinergic-adrenergic hypothesis of mood
disorders [20]. The high dose (2 mg/kg) of donepezil from our previous study [6], as well as this dose of galantamine and rivastigmine from the current study, tends to promote depression-like behavior in the FST. Our high dose of physostigmine (0.2 mg/kg) also was not therapeutic and was associated with depression-like behavior. Thus, the high dose findings from all four drugs in our two studies are consistent with the cholinergic-adrenergic hypothesis. Our low dose findings, however, suggest a novel modification to that hypothesis, given that they tended to be therapeutic-like rather than depression-like. Thus we suggest that the cholinergic-adrenergic hypothesis holds for relatively high doses of AChEIs, and by extension for relatively high synaptic concentrations of acetylcholine itself.

Since we tended not to observe with these four drugs robust antidepressant-like effects during the first (and very stressful) swim session, this may also be consistent with cholinergic-adrenergic functional opposition in another way: the adrenergic stress response may counteract the beneficial effects of these cholinergic drugs. As we have suggested previously for donepezil, associative learning processes may “gate” whether these drugs produce antidepressant-like effects in a given swim session [6]. In this scenario, the animal forms an association between the putatively highly aversive first swim session (FST1) and the interoceptive state produced by that dose of drug. When that dose of drug is presented again in subsequent swims (i.e., FST3 and FST6 in the current experiments) the animal tends not to exhibit an antidepressant-like effect due to this learned association. This learning effect may be particularly apparent for galantamine in the experiments described here.

Whereas male mice treated with donepezil, galantamine, physostigmine, or rivastigmine tend to no show strong antidepressant-like effects during the first swim session, the pattern in female mice may be different. In females treated with donepezil, we observed statistically significant antidepressant-like effects in FST1 (Experiment 7; Figure S4) with little drug modulation in subsequent swims of that experiment. This putative sex difference may represent divergent learning responses to the repeated forced swim experimental procedure used here, as well as a different behavioral reaction to the first and presumably most acutely stressful FST1. We also did not observe strong antidepressant-like responses with females in the two FSTs of Experiment 8 (Figure S5). One possibility is that female C57BL/6J mice are more resistant to the antidepressant-like effects of donepezil than males, which may have relevance to understanding the higher observed rates of MDD in women [36–38] and modeling these human sex differences in rodents.

Our findings in the current study add to the hypothesis that cholinesterase inhibition (and by extension, acetylcholine itself) has a u-shaped dose-response curve in the modulation of depression-related behaviors. We had previously shown this for donepezil [6], and here we demonstrate a similar relationship for galantamine, physostigmine, and rivastigmine. A similar u-shaped curve has been suggested for acetylcholine in substance abuse [39], as well as in memory-related tasks [40, 41]. In this scenario, having an “optimal” amount of cholinergic signaling may promote antidepressant-like behavior. If acetylcholine has a u-shaped dose-response curve in various behaviors, this is consistent with similar findings on the monoaminergic neurotransmitters serotonin, norepinephrine, and dopamine in various cognitive- and emotion-related behaviors, suggesting too much or too little signaling is pathological [42–45].

A perhaps surprising aspect of the hypothesis that reversible AChEIs have antidepressant-like properties is that these drugs are widely used in the clinical treatment of the age-related neurological disorder, Alzheimer’s disease, which is characterized by degeneration of the cholinergic basal forebrain [46,47]. Although MDD and Alzheimer’s disease are independent disorders, they are often comorbid and may share some aspects of their underlying pathophysiology [48]. Along these lines, the antidepressant citalopram has been shown to counteract cognitive impairment in Alzheimer’s to some extent [49], and AChEIs have been shown in some instances to have antidepressant qualities in elderly individuals with Alzheimer’s who were also suffering from MDD [50,51]. It largely remains to be determined if younger subjects suffering from MDD would benefit from AChEIs. Given our rodent data on AChEIs, one possibility is that these drugs might be effective in Alzheimer’s disease and other disorders such as MDD, at lower doses than they are typically now administered.

In conclusion, here we have demonstrated that the reversible AChEIs galantamine, physostigmine, and rivastigmine have antidepressant-like qualities in the mouse FST, extending our previous findings on donepezil [6] to show that a variety of these drugs have antidepressant-like effects. It is not clear if these preclinical findings in rodents extend to humans suffering from MDD, and it is also not well understood the extent to which associative learning processes modulate their effects in humans or rodents. We suggest that the fields of rodent behavioral neuroscience and clinical psychiatry (and neurology) should now investigate this topic in greater detail, to test whether AChEIs can be re purposed as antidepressants in human subjects, either in the presence or absence of comorbid Alzheimer’s disease.

Author statement

P.JF, PJH, BOW: Conceptualization; P.JF, PJH, BOW: Data curation; P.JF, PJH, BOW: Formal analysis; PJF, BOW: Funding acquisition; PJF: Investigation; PJF, PJH, BOW: Methodology; P.JF, BOW: Project administration; PJF, BOW: Resources; PJH, BOW: Software; BOW: Supervision; PJF, AG: Validation; PJF, BOW: Visualization; PJF, PJH, AG, BOW: Roles/Writing - original draft; PJF, PJH, AG, BOW: Writing - review & editing.

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

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