

The Combined Effects of Memantine and Fluoxetine on an Animal Model of Obsessive Compulsive Disorder

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Obsessive Compulsive Disorder (OCD) is currently treated with behavioral modification and psychotropic medications, with varying degrees of success. The most popular drugs for the treatment of OCD are the selective serotonin reuptake inhibitors (SSRIs). Another drug, the *N*-methyl-D-aspartate antagonist memantine, has recently been tested in the treatment of OCD. The present study investigates the effect of fluoxetine and memantine alone and in combination in a mouse model of compulsive behavior. In this model, compulsive scratching is induced by a subcutaneous injection of serotonin or a serotonin releasing agent, compound 48–80, in the back of the neck. The effects of the memantine and fluoxetine combination were found to synergistic, specifically as defined by an isobologram. The results of the present investigation suggest the potential of a more effective management of the symptoms of OCD.

Keywords: Obsessive-Compulsive Disorder, fluoxetine, memantine, scratching, synergism

Obsessive Compulsive Disorder (OCD) is a common neuropsychiatric disorder (Arnold & Richter, 2001; Mohammadi et al., 2004). The fourth edition text revision of the *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, 2000) defines OCD as consisting of obsessions, in which an individual fixates and ruminates on a certain idea or worry and/or compulsions. Compulsions may occur alone, without preceding obsessions, or may be triggered by obsessive thoughts, in which case they represent attempts by the individual to address obsessions by engaging in acts that may not be connected to the obsession in any realistic way (American Psychiatric Association, 2000).

OCD has been linked to a number of neuroanatomical abnormalities using neuro-imaging (Saxena, Brody, Schwartz, & Baxter, 1998). One study found anterior cingulate abnormalities to be an important factor in OCD (Yucel, Wood, Fornito, Riffkin, Velakoulis, & Pantelis, 2003). Another neuro-imaging investigation found people with OCD to have functional anomalies in neural circuitry connecting the basal ganglia, orbitofrontal cortex, and thalamus. These studies suggest that etiologically OCD has a considerable biological component (Saxena & Rauch, 2000). Thus, pharmacotherapy, in addition to behavior modification therapy, may be indicated in the treatment of OCD.

Selective Serotonin Reuptake Inhibitors

The Selective Serotonin Reuptake Inhibitors (SSRIs) are the only effective class of drugs used to treat OCD (Preston, O'Neal, & Talaga, 2001). Sertraline, paroxetine, fluoxetine, citalopram, fluvoxamine, and escitalopram are the most frequently prescribed SSRIs for treatment of OCD today. SSRIs act by preventing the reuptake of serotonin into the presynaptic neuron, thus more serotonin is left in the synapse to bind with postsynaptic receptors (Hjorth & Auerbach, 1995).

Fluoxetine was the first in a generation of SSRIs (Rossi, Barraco, & Donda, 2004). Many studies have tested the efficacy of fluoxetine in treatment of major depressive disorder and anxiety disorders, including OCD (Ferguson, 2001). SSRIs are generally considered to have better overall safety and tolerability than other antidepressants (Barbey & Roose, 1998). The most common side effects include sexual dysfunction, weight gain, palpitations, and nausea (Trindade, Menon, Topfer, & Coloma, 1998). There are also delayed effects after chronic administration of SSRIs, such as changes in the number of receptors for certain neurotransmitters and increased neurogenesis in certain brain regions (Santarelli et al., 2003).

Although fluoxetine helps relieve symptoms of OCD by potentially blocking the reuptake of serotonin, there is potential for further alleviation of these symptoms (Preston, O'Neal, & Talaga, 2001).

NMDA Antagonists

Memantine has been shown to slow cognitive decline in mild to severe Alzheimer's dementia (Tariot, Farlow, Grossberg, Graham, MacDonald, & Gergel, 2004). Memantine, known by its brand names as Ebixa, Namenda, Akatinol, and Axura, is a noncompetitive, moderate affinity,

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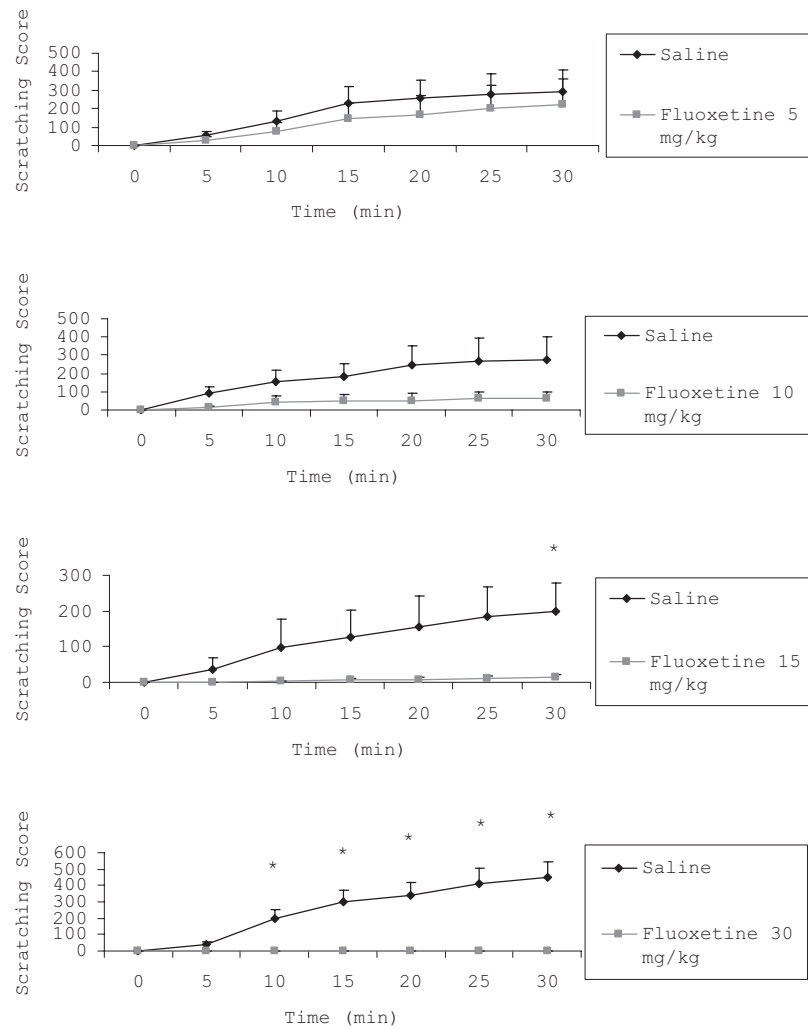


Figure 1. Effect of different doses of fluoxetine on serotonin-induced scratching. Asterisks indicate a significant difference from control ($p < .05$). The cumulative numbers of scratches at 30 minutes, expressed as a percent of control, were 76, 23, 7, and 0% for doses of 5, 10, 15, and 30 mg/kg, respectively.

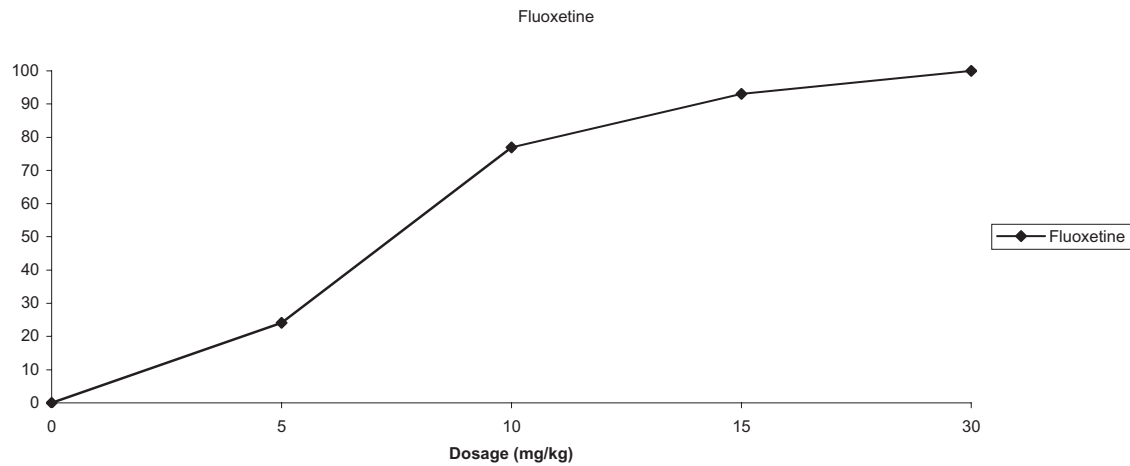


Figure 2. A dose response curve showing percent reduction in scratching after varying doses of fluoxetine.

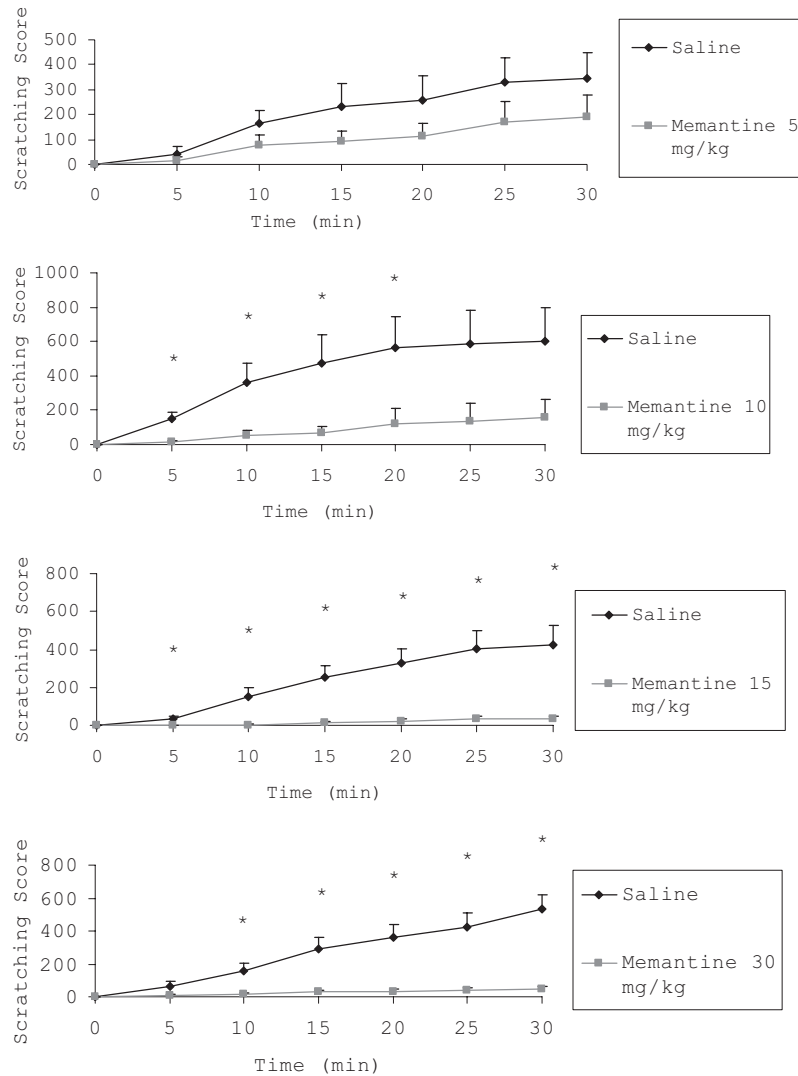


Figure 3. Effect of different doses of memantine on Serotonin-induced scratching. Asterisks indicate a significant difference from control ($p < .05$). The cumulative numbers of scratches at 30 minutes, expressed as a percent of control, were 55, 26, 8, and 9% for doses of 5, 10, 15, and 30 mg/kg, respectively.

voltage-dependent *N*-methyl-D-aspartate (NMDA) receptor antagonist (Rogawski & Wenk, 2003). In a 52-week study, memantine was well tolerated by patients with moderate to severe Alzheimer's dementia both with and without additional treatment with a cholinesterase inhibitor (Robinson & Keating, 2006).

NMDA receptors play an important role in learning and memory (Savonenko, Werka, Nikolaev, Zielinski, & Kaczmarek, 2003). Calcium influx at NMDA receptors is responsible for secondary cell death in patients with vascular dementia and head injury. Memantine prevents calcium influx that is caused by chronic over stimulation of NMDA receptors (Bernert & Turski, 1996). One study (Biegon, Fry, Paden, Alexandrovich, Tsenter, & Shohami, 2003) has shown memantine to be effective in preserving cognitive

function in rats after a closed head injury. Many researchers believe that this neuroprotective characteristic is the cause of prolonged cognitive function in individuals with other forms of dementia (Kornhuber, Weller, Schoppmeyer, & Riederer, 1994).

The present study, using a mouse model of OCD (Kuraishi, Nagasawa, Hayashi, & Satoh, 1995), examined whether there is a synergistic, rather than additive relationship, between NMDA antagonists and SSRIs by combining relatively low doses of both drugs that do not decrease compulsive behavior when administered alone.

Synergism was analyzed using an isobologram (Tallarida, 2001). In an isobologram, one dose of two different drugs that produce a given effect size is plotted on the y- and x-axis, respectively. A connecting line is then drawn be-

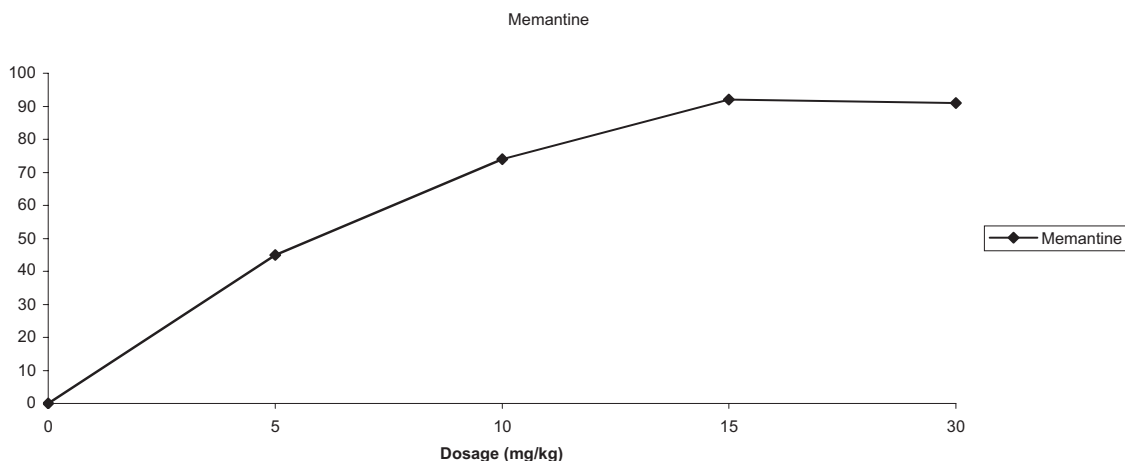


Figure 4. A dose response curve showing percent reduction in scratching after different doses of memantine.

tween these two points. A third point is then plotted on the graph showing the dose combinations of these two drugs necessary to produce the same effect size. Combination points below the dose connecting line are said to show synergism. Combination points along the dose connecting line are said to show additivity. Combination points above the dose connecting line are said to show subadditivity.

Method

Animals

Male Swiss Webster mice were used in this investigation. The mice weighed between 18 and 63 g depending on age. They were purchased from Taconic Laboratories in Germantown, NY. Mice were kept at 68 to 72 °F in a 12h/12h light–dark cycle. The mice were able to access water and food pellets in their cages ad libitum. The present investigation was approved by the animal subjects committee of Tufts Medical School in Boston.

Scratching Assay

Scratching has been established as an effective model for studying compulsive behavior in dogs with allergic

dermatitis (Dodman, Shuster, Nesbitt, Weissman, Lo, Chang, & Cottam, 2004). In that investigation, dextromethorphan, an NMDA antagonist, was shown to reduce scratching, self-chewing, and self-biting behaviors. Compulsive scratching can be induced in mice with an intradermal injection of either serotonin or compound 48–80, which, by causing degranulation of mast cells, releases serotonin in the skin of rodents (Kuraishi et al., 1995). Ascorbic acid was added to the injectate to protect serotonin from oxidation.

Each mouse was given an intraperitoneal injection of varying doses of fluoxetine and/or memantine in saline (0.9% NaCl) containing ascorbic acid (1 mg/ml). Control mice received an intraperitoneal injection of saline and ascorbic acid. The volume administered was 0.1 ml per 10 g of body weight.

Five minutes later, each mouse was injected subcutaneously, behind the neck, with 0.1 ml of 0.4 mg/ml serotonin or 0.1 ml of compound 48–80 1 mg/ml, in saline and ascorbic acid to induce scratching (Kuraishi et al., 1995). Each mouse was then placed individually in a 7 × 11 inch cage and paired with a mouse in a separate cage given saline pretreatment. The cumulative number of scratches was counted continuously using a manual counter. Cumulative

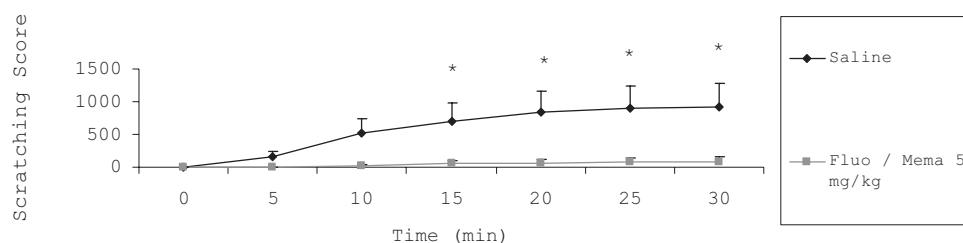


Figure 5. Effect of a combination of fluoxetine 5 mg/kg and memantine 5 mg/kg on serotonin-induced scratching. Asterisks indicate a significant difference from control ($p < .05$). The cumulative number of scratches at 30 minutes, expressed as a percent of control, was 9%.

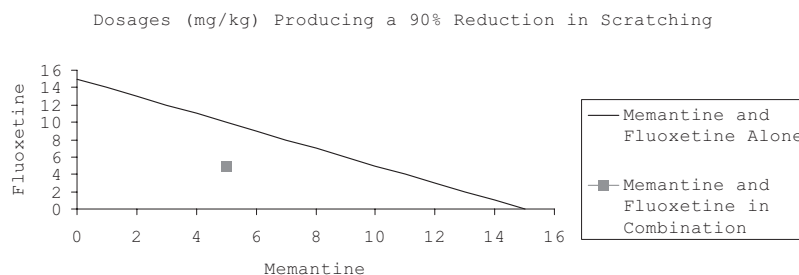


Figure 6. Isobologram displaying amount of drug required to produce a 90% reduction in scratching. The doses for memantine and fluoxetine alone are connected to establish a line beneath which all combinations of the two drugs producing a 90% effect size are considered to be synergistic. The total number of scratches at 30 minutes [\pm SEM] were: for 15 mg/kg of fluoxetine, 14.33 ± 5.74 ; for 15 mg/kg of memantine, 32.33 ± 14.60 ; and for a combination of 5 mg/kg of fluoxetine/memantine, 80.5 ± 73.64 .

scratches were recorded every 5 minutes for 30 minutes. Other behaviors noted by the observer included motor activity, sedation, licking, and rearing.

Statistical Analysis

Mean scratching scores for six pretreated mice were compared to their six respective saline controls using a series of matched samples two tail Student's *t* tests ($p < .05$). Additionally, cumulative scratches in mice injected with drug were expressed as a percent of scratches by saline control.

Results

The cumulative effects of increasing doses of fluoxetine on the scratching response to serotonin are shown in Figures 1 and 2. A significant reduction in compulsive scratching was observed only with fluoxetine doses of 15 and 30 mg/kg. Appreciable sedation was apparent only at the 30 mg/kg dose of fluoxetine.

Memantine by itself reduced scratching significantly only at doses of 10 mg/kg and higher (Figures 3 and 4). Appreciable sedation was apparent only at the 15 and 30 mg/kg doses of memantine.

The effect of subeffective doses of fluoxetine (5 mg/kg) and memantine (5 mg/kg) in combination is shown in

Figure 5. A significant reduction in scratching was found after a 15 minute lag time. An isobologram depicting the relationship between the dose of fluoxetine, memantine and the combination that reduce scratching by 90% is shown in Figure 6.

Additional experiments to demonstrate the effect of fluoxetine (10 mg/kg) and memantine (5 mg/kg) on scratching induced by compound 48–80, both alone and in combination, are shown in Figures 7, 8, and 9. Neither compound produced any significant reduction in scratching when administered alone but together a significant effect was noted with the combination after a 15-min lag time.

Discussion

Combined doses of fluoxetine and memantine required to produce a 90% reduction in scratching were much lower than the doses of either drug alone necessary to produce the same effect. These results indicate the possibility of a synergistic relationship between these two drugs in reducing compulsive scratching behavior. Sedation did not appear to be a contributor to this synergism as sedation was only observed at 30 mg/kg of fluoxetine as well as 15 mg/kg and 30 mg/kg of memantine. No sedation was observed in the combination experiments. There was also no major change in motor activity, rearing, or grooming, except at those doses that produced sedation.

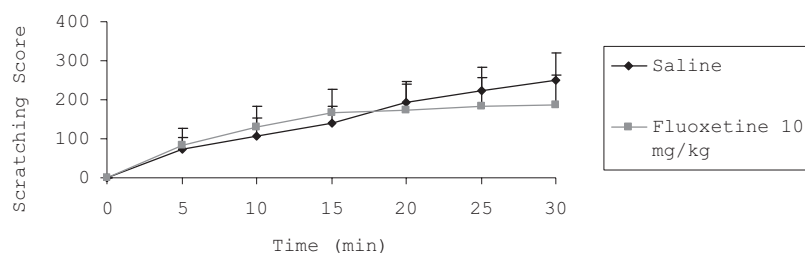


Figure 7. Effect of 10 mg/kg fluoxetine on 48–80 induced scratching. Cumulative scratching differences were not statistically significant at any 5 minute interval ($p < .05$). The cumulative number of scratches at 30 minutes, expressed as a percent of control, was 75%.

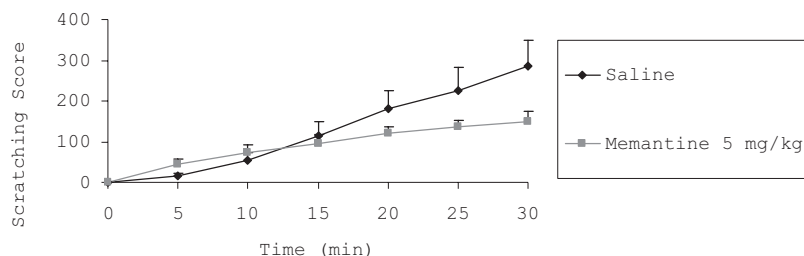


Figure 8. Effect of 5 mg/kg memantine on 48–80 induced scratching. Cumulative scratching differences were not statistically significant at any 5 minute interval ($p < .05$). The cumulative number of scratches at 30 minutes expressed as a percent of control, was 54%.

Compulsive scratching was induced in mice with a subcutaneous injection of either serotonin or compound 48–80, which, by causing degranulation of mast cells, releases serotonin in the skin of rodents (Kuraishi et al., 1995). In this model, the serotonin and 48–80 act as nonsystemic pruritogens.

Psychopharmacological drug synergism appears to occur most often when different neural mechanisms are affected by the drugs being investigated (Tallarida, 2001). In the present study, these mechanisms are most likely serotonergic and glutamatergic, because SSRIs increase the amount of serotonin in synapses and NMDA antagonists have been shown to block glutamate binding at NMDA receptor sites (Orgogozo, Rigaud, Stöffler, Möbius, & Forette, 2002).

The results of the present study appear to be consistent with a case report describing the successful use of memantine in treatment-resistant OCD (Poyurovsky, Weizman, Weizman, & Koran, 2005). In this case report, it is important to note that the NMDA antagonist was added to an SSRI, which was already being taken daily. Patients with OCD taking SSRIs generally should not be taken off their medication because of risk of exacerbation of their symptoms (Claxton, Li, & McKendrick, 2000). NMDA antagonists should be added to SSRIs to evaluate any additional beneficial effect.

Veterinary reports have also shown enhanced effects when an NMDA receptor blocking drug and serotonin reuptake inhibitor are employed concomitantly in spontaneously occurring canine equivalents of OCD (Maurer &

Dodman, 2007; Schneider, 2009). The effect of the two drugs appeared to be synergistic in this case.

It has recently been demonstrated that activation of 5HT₂ serotonergic receptors in the brain indirectly inhibits NMDA receptors by preventing the release of glutamate (Best & Regeher, 2008). In addition, *N*-acetyl cysteine, which inhibits the release of glutamate by acting on the cysteine-glutamate antiporter, has been used to ameliorate OCD in human patients (Pittenger, Krystal, & Coric, 2005). We have found that *N*-acetyl cysteine inhibits serotonin-induced compulsive scratching in mice. The basis for synergism between fluoxetine and memantine in our experiments seems to be that these agents use two different mechanisms to decrease the activation of central NMDA receptors by glutamate. Both decreased serotonergic activity and increased glutamatergic activity have been linked to the expression of impulsive behaviors (Pattij & Vanderschuren, 2008). We have also inhibited compulsive scratching in mice by direct activation of central 5HT_{2C} receptors with analogs of psilocybin (Sard et al., 2005).

The effects of memantine augmentation in treatment resistant patients with OCD has also been studied by Abujaoude, Barry, and Gamel (2008). Results of this investigation show a decrease in scores on the Yale-Brown Obsessive Compulsive scale when memantine is given to patients already taking a SSRI. The authors reported that side effects were mild and transient. No participants withdrew from the experiment as a result of side effects. While the study by Abujaoude, Barry, and Gamel (2008) also supports memantine augmentation in treatment resistant OCD, further testing is needed to analyze other behavioral endpoints.

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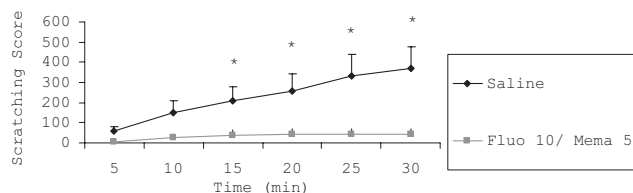


Figure 9. Effect of 10 mg/kg fluoxetine in combination with 5 mg/kg memantine on 48–80 induced scratching. Asterisks indicate a significant difference from control ($p < .05$). The cumulative number of scratches at 30 minutes expressed as a percent of control, was 11%.

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