Ciproxifan, an H_3 Receptor Antagonist, Improves Learning and Memory in the APP Mouse Model of Alzheimer's Disease.

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Running Head: H_3 antagonists and Alzheimer's Disease

Mice that express the mutant form of the human amyloid precursor gene associated with early-onset familial Alzheimer's disease demonstrate memory deficits and amyloid plaques. We show here that ciproxifan, a prototypical antagonist of H_3-type histamine receptors, alleviates two types of learning and memory impairments in such mice. These data support the idea that modulation of H_3 receptors represents a viable therapeutic strategy in the treatment of Alzheimer's disease.

Over the past decade, preclinical research has identified the H_3 histamine receptor as a possible target for cognitive-enhancing drugs (1, 2). The H_3 receptor exists as a presynaptic autoreceptor that is expressed in relatively high densities in brain regions associated with memory function, such as the frontal cortex and hippocampus (3). Antagonism of the receptor leads to the release of histamine as well as neurotransmitters involved in learning and memory, such as acetylcholine and dopamine (4 - 6). Moreover, H_3 antagonists can generate electrical activity in the brain that predicts new learning (7). On a behavioral level, drugs that act as H_3 antagonists, such as the prototypical imidazole-containing compounds, thioperamide and ciproxifan, have been shown to improve memory function in several tasks – in normal rats and mice, as well as in animals treated with anti-cholinergic or anti-glutamatergic drugs (8 - 11).

The ability of H_3 antagonists to enhance memory in normal animals and in pharmacological models of memory impairment raises the possibility that such compounds may represent an effective treatment strategy for Alzheimer's disease. As a way of addressing this possibility, we tested the effects of the H_3 antagonist, ciproxifan (8), on learning and memory deficits observed in the amyloid precursor protein (APP) transgenic mouse model of Alzheimer's disease. Developed by Hsiao and colleagues (12), APP mice express a mutant form of the human APP gene associated with early-onset, familial Alzheimer's disease. These mice exhibit a phenotype that includes deficits in spatial learning and memory and object recognition (12,13), as well as the formation of amyloid plaques with increasing age.

In the first study, we tested the effects of ciproxifan on spatial memory and locomotor activity in APP mice and wild-type (WT) littermates of both genders at 12-14 months of age. Approximately half of the mice of each genotype received intraperitoneal injections of ciproxifan (3 mg/kg) and the other half received injections of saline. The dose of ciproxifan chosen for study was based on previous research demonstrating the ability of this dose to improve memory in normal rats (9). Mice received daily injections over the course of three weeks and on behavioral testing days received such injections 30 minutes prior to testing.

During the second week of treatment, all mice were tested for spatial learning in the swim maze task. Ciproxifan improved performance in APP mice over the course of the training trials (**Fig. 1**). There was a statistical interaction between ciproxifan treatment and APP genotype (Interaction term: F (1, 34) =

6.93, $p < .01$) on the time to find the platform measure. APP mice treated with saline took longer to find the hidden platform in the test pool relative to wild-type mice treated with saline on days 1, 4, & 5 of training (**Fig. 1a**). On the latter two days, APP mice treated with ciproxifan took less time to find the platform than APP mice treated with saline (**Fig. 1a**). Also during the training trials, there was a treatment by genotype interaction (Interaction term: F (1, 34) = 4.46, $p < .04$) as well as an overall training day effect (F (4, 136) = 9.7, $p < .0001$) on the average distance from the platform measure. During the last four days of training, APP mice treated with saline tended to swim farther away from the platform in comparison to APP mice treated with ciproxifan and WT mice treated with saline (**Fig. 1b**). It should also be noted that there was trend towards a significant treatment x genotype interaction for the distance travelled before finding the platform (Interaction term: F (1, 34) = 4.46, $p = .11$ (two-tailed))(**Supplemental Figure 1a**). Finally, ciproxifan increased swimming speed in APP mice (Interaction term: F (1, 34) = 6.54, $p < .02$)(**Supplemental Figure 1b)**. In this case, the most consistent group difference over the last four days of training was between the APP mice treated with ciproxifan and APP mice treated with saline. Unlike the results for the other measures, APP mice treated with saline did not differ from WT mice treated with saline during the last four days of training. This distinct pattern of data suggests that group differences in swim speed do not account completely for the significant differences observed on the other measures.

One hour after the final training session, all mice were placed in the test pool for one minute without the platform. During this probe trial, APP mice treated with ciproxifan made as many crossings of the former platform site as did the WT mice treated with saline and significantly more than the APP mice treated with saline (Interaction term: F (1, 34) = 4.68, $p < .04$)(**Fig. 1c**). Moreover, when the amount of time spent in an area of the pool that included the former platform was measured, the same pattern of results emerged. APP mice treated with ciproxifan spent as much time as each group of WT mice in the zone that formerly contained the platform. In contrast to these groups, APP mice treated with saline spent significantly less time in this area (Interaction term: F (1, 34) = 6.45, $p < .02$)(**Fig. 1d**). Mice were also tested 48 hours after the last training trial and the effects of drug and genotype were not significant at that time (**Supplemental Fig. 2**).

A separate cohort of 12 – 14 month old APP and WT mice were tested on a novel object recognition (NOR) task. Ciproxifan or saline was injected into the mice 30 minutes prior to testing. Ciproxifan improved NOR in APP mice (Interaction term: F (1, 20) = 11.8, $p < .003$) (**Fig. 2a**). APP mice spent significantly less time exploring a novel object relative to a familiar one in comparison to all other groups, including the APP mice treatment with ciproxifan. The preference of this latter group of mice for the novel object did not differ from the WT mice treated with saline. These results were not due to changes in general exploration since neither genotype or drug treatment altered the total time spent exploring both objects during testing (**Fig. 2b**).

Finally, we assessed locomotor activity in the same cohort of APP and WT mice used in the swim maze test. This testing was performed one week prior to swim maze testing. Mice were tested for one hour a day for five days. APP mice treated with saline were significantly more active than WT mice treated with saline (Genotype x treatment x time interaction: F (11, 385) = 2.0, $p < .03$)(**Fig. 2c**). Ciproxifan reversed this effect. APP mice treated with ciproxifan were significantly less active than the APP mice treated with saline and were not different from the WT mice treated with saline during the first 25 minutes of testing.

These experiments are the first to demonstrate the ability of an H_3 antagonist to alleviate memory deficits and hyperactivity in a transgenic mouse model of Alzheimer's disease. The capacity for ciproxifan to improve cognitive and behavioral outcomes across multiple tests provides robust support for the pursuit of H_3 antagonism as a therapeutic strategy in the treatment of Alzheimer's disease. Current palliative treatments for Alzheimer's disease include acetylcholinesterase inhibitors and NMDA antagonists, yet these treatments possess only limited efficacy and significant side effects. Receptor binding data has shown that H_3 receptor densities are preserved in the brains of people with Alzheimer's despite disease progression (5), indicating that the target for H_3 antagonists remains a viable one throughout the disease process. Therefore, antagonism of H_3 receptors may represent an alternative pathway to cognitive enhancement or at least an approach that can be coupled with current treatments (14).

It is likely that ciproxifan's efficacy in the current study is related to its ability to enhance neurotransmitter release in the frontal cortex and hippocampus, and to generate electrophysiological activity predictive of learning. It will be important for future studies to test these ideas as well as to determine if longer term treatment with H_3 antagonists can modify pathophysiological processes (e.g. plaque formation, synapse loss) observed in the APP mouse model (15).

ACKNOWLEDGEMENTS: This work was supported by National Center for Research Resources Grant 2P20 RR16481 and National Institute of Mental Health Grant R15 MH076788 to M.E.B. We appreciate the assistance of Dr. Patrick J. Schultheis with the genotyping of the mice.

AUTHOR CONTRIBUTIONS: M.E.B. conceptualized and designed the project and wrote the manuscript. N.N.D. helped in designing the object recognition experiment and conducted the experiment. M.S.G. helped to design the entire project, genotyped the mice, conducted the swim maze and amyloid plaque experiments, and helped to write the manuscript.

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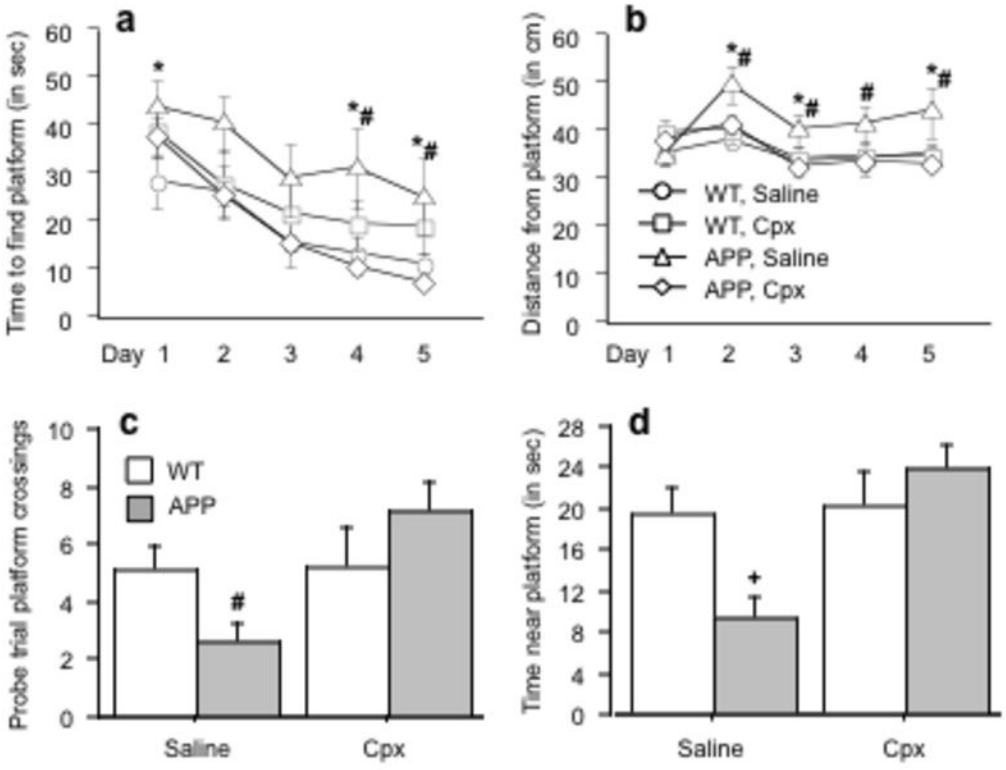
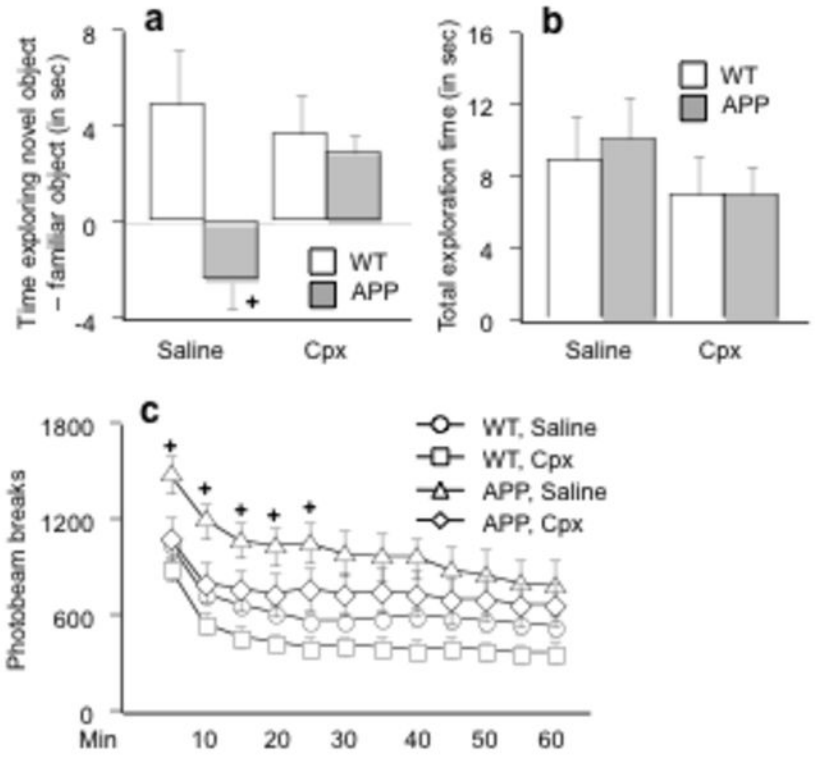
Figure legends:

Figure 1 Ciproxifan alleviates learning deficits exhibited by the APP mice in the swim maze task. (**a**) APP mice treated with saline took longer to find the escape platform than saline-treated WT mice on days 1, 4, & 5 of training, as indicated by *****, and APP mice treated with ciproxifan (3.0 mg/kg) on days 4 & 5 of testing, as indicated by #. (**b**) APP mice swam, on average, a greater distance away from the platform in comparison to saline-treated WT mice on days 2, 3, & 5 of training, as indicated by *****, and APP mice treated with ciproxifan on training days 2 – 5, as indicated by the #. There was an overall training day effect, indicative of learning across training days. (**c**) APP mice treated with saline displayed fewer crossings of the former platform location during the first probe trial in comparison to the APP mice treated with ciproxifan as indicated by #. (**d**) APP mice treated with saline spent less time near the former location of the platform during the probe trial in comparison to all other groups including the APP mice treated with ciproxifan as indicated by **+**. All data represent means $\pm$ s.e.m. All symbols represent statistically significant differences ($p < .05$, two-tailed).

Figure 2 Ciproxifan alleviates the novel object recognition deficit and hyperactivity observed in APP mice. (**a**) APP mice treated with saline spent less

time exploring a novel object relative to the time spent exploring a familiar one when compared to all other groups, including the APP mice treated with ciproxifan as indicated by +. (**b**) The total time spent exploring both objects during the test trial did not differ between groups. (**c**) APP mice were significantly more active during the first 25 minutes of activity testing relative to all of the other groups, including the APP mice treated with ciproxifan as indicated by **+**. All data represent means $\pm$ s.e.m. All symbols represent statistically significant differences ($p < .05$, two-tailed).


a
Time to find platform (in sec)
60
50
40
30
20
10
0
Day 1 2 3 4 5
*
*#
*#
b
Distance from platform (in cm)
60
50
40
30
20
10
0
Day 1 2 3 4 5
*#
*#
#
*#
WT, Saline
WT, Cpx
APP, Saline
APP, Cpx
c
Probe trial platform crossings
10
8
6
4
2
0
WT
APP
#
Saline
Cpx
d
Time near platform (in sec)
28
24
20
16
12
8
4
0
+
Saline
Cpx


a
Time exploring novel object – familiar object (in sec)
8
4
0
-4
WT
APP
+
Saline
Cpx
b
Total exploration time (in sec)
16
12
8
4
0
WT
APP
Saline
Cpx
c
Photobeam breaks
1800
1200
600
0
Min
10
20
30
40
50
60
WT, Saline
WT, Cpx
APP, Saline
APP, Cpx