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Adverse effects of dextromethorphan on the spatial learning of rats in the Morris water maze

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Abstract

The effects of the non-competitive NMDA receptor antagonist dextromethorphan on spatial learning were assessed using the Morris water maze. Dextromethorphan was administered to 4 groups of rats in 10, 20, 30, and 40 mg/kg doses. An additional group of rats was administered saline to serve as a vehicle control group. Dextromethorphan impaired learning dose dependently in the initial training phase of the experiment. During the probe trial, dose-dependent performance deficits were noted in the first 15 s of the trial only. Search strategy differences between the lowest and highest dose groups were also observed during the probe trial. During the reversal training phase, when the platform was moved to a new location, the dose-dependent impairment was seen again, but the 40 mg/kg group perseverated to the former location longer than the other groups. A cued control trial indicated that in addition to the learning impairment produced, the highest dose of dextromethorphan may also impair sensory-motor coordination.

Keywords: Dextromethorphan; NMDA receptor; Spatial learning; Memory; Water maze

1. Introduction

Since its discovery over a decade ago, the NMDA receptor, a subclass of glutamate receptors, has been shown to be involved in a multitude of physiological processes. Many investigations have shown that over-activation of these receptors can contribute to neurodegenerative processes underlying ischaemia (Ohno et al., 1992; Steinberg et al., 1993), epilepsy (Feeser et al., 1988; Löscher and Hönack, 1993; Schmitt et al., 1994), and hyperglycemia (Schmitt et al., 1993). Researchers have therefore focused their efforts on identifying ligands that will antagonistically bind to the NMDA receptor, preventing or attenuating the brain damage incurred after such insults. However, there is substantial evidence that the NMDA receptor also plays a significant role in synaptic plasticity (Cotman and Iversen, 1987; Morris et al., 1986). NMDA receptor-mediated synaptic plasticity is believed to be the basis of certain commonly studied learning and memory processes involving the hippocampus (Morris, 1988). Some forms of long-term potentiation, a putative physiological substrate underlying learning and memory, have been shown to be NMDA receptor-dependent (Morris et al., 1986, 1989). Furthermore, many studies indicate that NMDA receptor antagonists that interfere with long-term potentiation also disrupt performance on learning tasks, particularly tasks involving spatial memory (Butelman, 1989; Davis et al., 1988; Malenfant et al., 1990). Because the NMDA receptor is involved in both synaptic plasticity and neurodegenerative process, it is important that potential anticonvulsant/neuroprotective drugs targeting this receptor be evaluated in terms of their interference with normal learning and memory processes.

Dextromethorphan, a non-opioid antitussive, has recently been shown to have both anticonvulsant and neuroprotectant properties (Trube and Netzer, 1994). It is a particularly attractive candidate for clinical use since it has been dispensed as a non-prescription drug for over 40 years and is known to have a wide margin of safety (Schmitt et al., 1994). Antagonists having neuroprotective and or anticonvulsive properties have been identified for the glycine and Mg^{2+} sites (Muir and Lees, 1995), as well as the phencyclidine (Mallamo et al., 1994) binding site on the NMDA receptor complex. Dextromethorphan's neuroprotectant and anticonvulsant properties appear to result from the binding of the drug to the NMDA receptor ion pore (Trube and Netzer, 1994). Recently, dextromethor-

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phan has also been shown to prevent the induction of long-term potentiation in vivo (Krug et al., 1993).

Although recent evidence indicates that dextromethorphan has the potential to be an effective neuroprotectant/anticonvulsant in animal models, few studies have formally investigated the effects of the drug on learning and memory processes. Investigators have reported that dextromethorphan (Murata and Kawasaki, 1993) and its metabolite dextrorphan (Sierocinska et al., 1991) impair passive avoidance learning in rats. Given that many other NMDA receptor antagonists affect learning (Ylinen et al., 1995), and that dextromethorphan is an antagonist at the NMDA receptor, this study was undertaken to examine the effects of the drug on spatial memory function in rats. We used the Morris water maze (Morris et al., 1986) to assess the effects of four different doses of dextromethorphan on spatial learning in the rat.

2. Materials and methods

2.1. Subjects

Fifty, three month-old male Sprague-Dawley rats (Charles River, MA), weighing between 350 and 425 g, were used in these experiments. All animals were housed individually, maintained on a 12 h light/dark cycle (6:00 am/6:00 pm, respectively), and had free access to food (Purina rat chow) and water during the course of the study.

2.2. Apparatus

A circular, aluminum water maze measuring 183 cm in diameter and 61 cm in height was employed as the test apparatus. The tank was filled with water at 18°C to a height of 30 cm. Non-toxic, black temper-paint (Dry Temp, Palmer Paint Products) was used to make the water opaque. A circular escape platform with a diameter of 10 cm was submerged 1 cm beneath the surface of the water in the center of one quadrant of the water tank, 41 cm from the edge. The tank was located in a small room with a variety of extra maze stimuli (e.g., posters) located on the walls.

A video tracking system (PolyTrack Video Tracking System, San Diego Instruments, San Diego, CA) was used to record the movement of the rats within the maze, latency to the escape platform, and distances swum during the trial. A computer located outside of the test area monitored the animals' swim paths via a video camera mounted above the water tank. For data analysis, the computer divided the water tank into four equal quadrants, one of which contained the platform, to track the rats' performances in different sections of the maze.

Dextromethorphan HBr (Sigma, MO) was dissolved in 0.9% saline to the appropriate concentrations of 10, 20, 30, and 40 mg/2 ml. Ten animals were randomly selected to participate in each of the four dextromethorphan doses, as well as ten animals who received control injections of saline. All injections were given intraperitoneally at 2 ml/kg.

2.3. Procedure

On each day of the experiment, injections were given to the animals at least 15 min prior to their performance in the maze. Each animal performed the task within 45 min of receiving its injection.

To assess the rat's ability to learn the location of the platform, the first phase of the experiment consisted of seven days of training. There were three trials in the maze on each day of training. The rats were randomly placed in one of three non-platform quadrants within the maze, approximately 5 cm away from and facing the edge of the tank. The animals were given 60 s to find the escape platform. If they found the platform within 60 s, they were allowed to remain on the platform for 30 s, after which they were removed from the tank and placed under a heat lamp for a 30 s intertrial interval. Animals that did not find the platform after 60 s were placed there by the experimenter so that all animals spent an equal amount of time on the platform. Between trials, feces were removed from the tank and the water was stirred to disrupt any possible olfactory cues. After the 30 s intertrial interval, the rats were placed into the tank for their next trial. Latency to the escape platform, as well as a record of each animal's swim path, were recorded on the computer for each trial. After three trials, an animal was removed from the tank, toweled dry, and allowed to remain under a heat lamp for 5 min before being returned to its home cage.

Consistent with previous research indicating that rats receiving injections of NMDA receptor antagonists tend to jump off the escape platform once they find it (Ylinen et al., 1995), we did notice that in the early training trials our dextromethorphan-treated animals did not stay on the platform. Rats that strayed from the platform during the 30 s waiting period were manually guided back to the platform by an experimenter.

After initial training, a probe trial was given on the first trial of the eighth day. The probe was conducted without the escape platform in the tank. The rats swam in the tank for 60 s during the probe trial, and were then removed for the next phase of training. The purpose of the probe trial was to record the animal's performance without the influence of chance encounters with the platform, as well as to employ a measure of maze learning that did not punish the animal for not finding the platform (dwell time in quadrant versus escape latency).

The second and third trials of day 8, as well as the three trials on day 9, served to retrain the rats to the location of the platform, to prevent animals from extinguishing the platform search behaviors established by the initial training. The escape platform was located in its original location in the water maze, and all other parameters were the same as for the initial training trials.

After retraining, the next phase of the experiment consisted of four days of reversal training to assess the rats' ability to learn a new escape platform location within the maze. The platform was moved to the quadrant opposite from its original location. All other conditions were the same as in the initial training period.

The final day of the experiment consisted of three cued trials which served as a control for any sensory-motor impairments caused by the drug. On each cued trial, the platform was raised 2 cm above the waterline and marked with an 18 cm tall, white 2-dimensional flag in the shape of a cross (viewed from above). Each of the four panels of the flag measured 5 cm in length by 4 cm high. The location of the platform was moved to a different, non-target quadrant for each trial. Escape latency was measured as before, and the rats were allowed a maximum of 60 s to reach the platform. All 50 animals were given one trial at each location before the platform was moved to a new location. Thus, each animal received approximately 1 h of rest between its three cued trials.

3. Results

Preliminary inspection of the raw data indicated that the variances among groups were non-homogeneous. A plot of residual variances indicated that the variance increased as the drug dose increased, especially during the reversal training phase of the experiment. Therefore, all the data were log-transformed (base 10) for the analyses of escape latencies. The Statistical Package for the Social Sciences was used for all statistical analyses.

All five groups of animals showed a progressive decline in escape latency over the first seven training days (Fig. 1). Statistical analyses were performed on the average of the rats' three escape latencies per day. To analyze the group main effect, and thus any dose dependent effects of the drug on spatial learning, dose was coded as a polynomial contrast and tested for the highest level of trend in the data. A linear trend (contained in the first degree of freedom) would indicate that the drug produced a dose dependent effect, while a cubic trend could indicate a threshold for effect. A two-way, mixed-design analysis of variance on log escape latency revealed only a significant linear trend for dose, $F(1,45) = 28.50$, $P < 0.01$. The day main effect was also significant, $F(6,270) = 86.14$, $P < 0.01$, but the dose by day interaction was non-significant, $F(6,270) = 1.60$, $P > 0.05$.

To evaluate the effect of the drug on the probe trial, a one-way, between-subjects analysis of variance was performed, and revealed no significant differences among doses with respect to time spent in the target quadrant, $F(12,180) = 0.91$, $P > 0.05$. During the probe trial however, we observed that the animals' search strategies appeared to change over time, in terms of the proportion of time spent in the target quadrant to the opposite quadrant. Therefore, the duration of the probe trial (60 s) was divided into four 15 s quartiles for further analysis, and a ratio score of the time spent in the target quadrant to the opposite quadrant was calculated for each quartile, then log transformed (1 s was added to all dwell times to avoid divide by zero errors). A large ratio score would indicate that an animal spent more time in the target quadrant than the opposite quadrant during the probe trial, and a low ratio score would indicate more time spent in the opposite quadrant. Thus, this score provides a measure of search strategy during the probe trial. A two-way, mixed-design analysis of variance (dose by quartile) on the ratio score revealed a significant main effect for quartile, $F(3,135) = 3.73$, $P < 0.05$, and a significant dose by quartile interac-

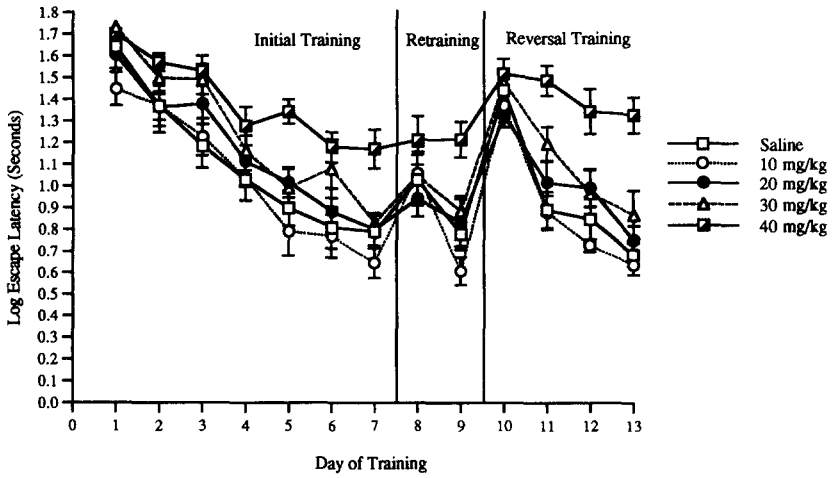

Fig. 1. Escape latencies for all phases of experiment. Number of seconds to escape platform was log transformed. Group means ± S.E.M. are shown. Note that on day 8, the plotted point is an average of two retraining trials, not three, because the first trial of that day was the probe (see Methods). Drug was administered 15 min prior to entry in the water maze. A significant linear trend of the dose main effect was obtained for the initial training phase, $F(1,45) = 28.50$, $P < 0.01$, and the reversal training phase, $F(1,45) = 48.44$, $P < 0.01$. The reversal training phase also produced a significant quadratic effect for dose, $F(1,45) = 13.36$, $P < 0.01$.

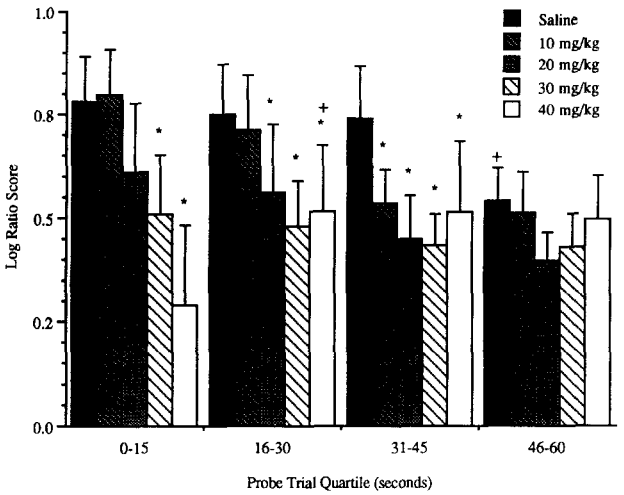

Fig. 2. Search strategy during probe trial. The 60 s trial was divided into four 15 s quartiles for analyses. A ratio score indicating search strategy was calculated by dividing dwell time in target quadrant by dwell time in quadrant opposite to target. Data were then log transformed to correct for non-homogeneous variances. Results are shown as means ± S.E.M. (* indicates significant Fisher's LSD result, $P < 0.05$ compared to saline group within same quartile; + indicates $P < 0.05$ compared to same group's performance in previous quartile).

tion, $F(12,135) = 1.84$, $P < 0.05$. Fig. 2 shows the specific pair-wise differences (Fisher's Least Significant Difference) obtained from this analysis.

To evaluate the reversal phase of the study, a two-way, mixed-design analysis of variance (dose by training day) was performed on the escape latency data. As before, the linear trend was significant, $F(1,45) = 48.44$, $P < 0.01$. However, the quadratic effect was also significant in this phase, $F(1,45) = 13.36$, $P < 0.01$, possibly due to the large difference between the 40 mg/kg dose and the other doses. The cubic effect was non-significant, $F(1,45) = 0.00$, $P > 0.05$. The day main effect was significant, $F(3,135) = 72.37$, $P < 0.01$, and the dose by day interaction was non-significant, $F(3,135) = 1.03$, $P > 0.05$.

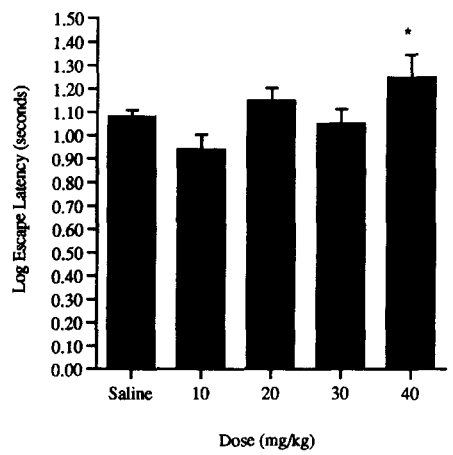

Fig. 3. Cued trial escape latencies. Data are presented as group means ± S.E.M. (* indicates significant Fisher's LSD result, $P < 0.05$, between 40 mg/kg group and 10 mg/kg group).

To test for any potential sensory-motor effects the drug might have on performance, the log-transformed data from the cued trials were averaged for each animal, subjected to a oneway, between-subjects analysis of variance and found to be significant, $F(4,45) = 3.45$, $P < 0.05$ (Fig. 3). Subsequent Fisher's Least Significant Difference pair-wise comparisons indicated that the animals receiving the 40 mg/kg dose had significantly slower escape latencies than the 10 mg/kg group ($P < 0.01$).

4. Discussion

Several interesting findings emerged from these experiments. First, administration of the NMDA receptor antagonist dextromethorphan impaired acquisition of the Morris water maze task in rats. The first seven days of training required that the animals locate the hidden platform using extra-maze spatial cues learned on previous trials to navigate the water maze. Although animals in all groups became more efficient at finding the platform (as evidenced by decreased escape latencies), the significant linear trend obtained indicated that performance was dose dependently impaired by dextromethorphan. This result is consistent with those of other investigators using NMDA receptor antagonists (e.g., MK-801 and AP-5) in the Morris water maze (Morris et al., 1986; Ylinen et al., 1995). However, it should be noted that the initial training performance of rodents in the water maze is generally dependent upon two factors: (a) habituation of perimeter preference and (b) learning the location of the escape platform. Previous research has indicated slower perimeter habituation in NMDA receptor antagonist-treated rats (Venable and Kelly, 1991), and we did note that our dextromethorphan-treated animals appeared slow to move towards the center of the maze. Thus, some of the impairment seen on the first training days is likely attributable to a combination of the two factors, and not a spatial learning deficit per se. Another concern is that latency to platform may be confounded by swim speed, thus masking deficits attributable to learning impairment. However, we observed the same effects on performance in the maze when distance swum was used as the dependent measure, and because distance swum to platform is theoretically less subject to motor effects, we believe that the escape latency data support our conclusion of a learning impairment.

The initial analysis of the probe trial data yielded no significant differences among the groups in the time spent in the target quadrant; however, the ratio score revealed a previously unreported finding that may pertain to future investigations. Traditional evaluation of dwell time in quadrant collapsed over the duration of the probe trial was not sensitive to the impairments in search behavior produced by dextromethorphan in this study. The finding that the ratio score changes over time in the maze may represent an adaptive search strategy normally seen in rats. An

animal limiting its search to an area just surrounding the former location of the platform would be unlikely to find the escape platform if it was moved to a different location in the maze. In the same situation, an animal that deviated from the previous learned location and searched elsewhere would be more likely to find the new location of the platform. Our findings suggest that changes in search strategy over the course of the probe trial may reveal important behavioral changes not readily seen with other analyses.

Analysis of the probe trial data using this method revealed both a marked difference in performance during the first 15 s of the trial and a differential change in search strategy among the groups. During the first 15 s a dose-dependent trend was observed, where the rats in the saline group had the highest ratio score and rats receiving higher doses had a lower ratio score. The ratio score for the saline group remained high throughout the first 45 s of the trial and dropped off significantly in the last 15 s, indicating a broadening of the search pattern. Although not reaching statistical significance, rats in the 10 and 20 mg/kg group showed a similar trend with the change in search strategy beginning in the last 30 s of the trial. Animals in the 30 mg/kg group showed a consistent search strategy that changed little throughout the trial. Rats receiving the 40 mg/kg dose, on the other hand, showed a strategy opposite to that of the saline group. These animals had a significantly higher ratio in the last 45 s compared with their ratio score in the first 15 s. Although it is possible that a motor impairment could explain even the time-dependent change in strategy observed in the 40 mg/kg group, this is unlikely given that the 30 and 40 mg/kg groups never acheived a ratio score similar to that of the saline animals in the first three quartiles (see Fig. 2).

Previous studies have shown that animals with hippocampal damage tend to perseverate previously learned behaviors (Jones and Mishkin, 1972; Mitchell et al., 1993). Reversal training on days 10–13 was employed to test the possibility that antagonism of the NMDA receptor with dextromethorphan would produce a similar effect. An evaluation of the escape latencies during retraining (Fig. 2) indicated that rats receiving injections of dextromethorphan took longer to retrain to the new platform location, suggesting possible perseverative responding to the old location. This finding was particularly evident at the 40 mg/kg dose.

The cued trials were used as a control for possible sensory-motor impairments. Because the platform was raised above the level of the water and its location was changed from trial to trial, escape latency during this phase was not memory-dependent. Differences in performance could therefore indicate a problem in motor coordination, visual impairment, and/or vestibular dysfunction. The results showed that the animals in the 40 mg/kg group had significantly longer escape latencies than the animals in the 10 mg/kg group. Unpublished studies in our laboratory have shown that if dextromethorphan is injected i.p. at twice the concentration of the highest dose used in the current study, severe ataxia and pronounced unidirectional circling behavior are seen in some animals. Some of the rats in the high dose group may have had milder motor impairments, but none of the animals receiving the lower doses did. Other investigators have reported similar results to ours using other NMDA receptor antagonists such as MK-801 (Danysz et al., 1994; Murata and Kawasaki, 1993). We have also noticed that this higher concentration produces clonic convulsive episodes in some rats, several minutes after injection. Löscher and Hönack (1993) previously reported the induction of clonic convulsive activity in two seizure kindled rats at 60 mg/kg, and there is one report in the human clinical literature of increased seizure frequency produced by dextromethorphan (Fisher et al., 1990). These findings suggest that some of the effects seen in this report for the high dose group could be attributed to something other than memory impairment, and this possibility should be studied further. However, it is important to note that while the high dose group's cued latency was significantly longer than the 10 mg/kg group, their performance did not differ from the saline controls. Further, we did not observe any unusual motor impairments in the rats receiving lower doses.

Our results suggest that dextromethorphan impairs spatial learning in the Morris water maze in a dose-dependent manner, and also produces perseverative responding to previously learned information. One potential implication for this result is that clinicians interested in dextromethorphan's anticonvulsive or neuroprotective qualities should consider its possible detrimental effect on memory, as well as the potentially dangerous side-effects discussed above. We have also shown that rats' performance in the Morris water maze during the probe trial changes significantly over time. Given that some investigators use even longer probe trials than in our current study (up to 2 min; Bannerman et al., 1994), important behavioral changes may be overlooked if time-dependent search strategies are not considered as mediating factors.

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