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Short communication

Metrifonate improves working but not reference memory performance in a spatial cone field task

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Abstract

The effects of metrifonate (3, 10 and 30 mg/kg, p.o.) on the working and reference memory performance of the rat were assessed in a spatial cone field task. The highest dose of metrifonate (30 mg/kg) improved the working memory performance, whereas none of the doses affected the reference memory performance. Other parameters of spatial discrimination performance were not affected by metrifonate treatment. The present results suggest that metrifonate has cognition-enhancing properties which are likely to be related to aspects of (spatial) working memory. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Metrifonate is a so-called 'second generation' acetylcholinesterase inhibitor. Its cognitive therapeutic potential has already been extensively investigated in preclinical animal studies (Schmidt et al., 1997), where it was found to improve learning and memory after acute as well as repeated oral treatment in both young and old rats. Metrifonate has also been shown to have a cognition-enhancing effect in patients with Alzheimer's disease (Cummings et al., 1998; Morris et al., 1998). This indicates that animal models can be used to predict the clinical efficacy of cognition-enhancing drugs.

The aim of the present study was to further characterize the cognition-enhancing properties of metrifonate in rats in a spatial cone field task. This task measures spatial discrimination learning but can also be used to distinguish between (spatial) working- and reference memory (e.g., van der Staay et al., 1990a). In this spatial task, rats have to learn to discriminate between baited and non-baited cones (four out of 16) by using spatial cues. The most efficient food search strategy is to visit the four baited cones only once. It is assumed that the list of cones visited is held in the working memory. This aspect of memory is required to avoid making revisits to baited locations (Olton et al., 1980). While trial-dependent relevant information is kept in the working memory, trial-independent information, such as the (fixed) location of the baited locations (i.e., cones), is held in the reference memory (Barnes, 1988). This memory component is assumed to be used to distinguish between baited and non-baited cones.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the local ethics committee for animal experiments of Maastricht University and met governmental guidelines. Thirty-five 12-month old male Wistar rats (Janvier, France) were used. The animals were housed individually in standard Makrolon cages on sawdust bedding in an air-conditioned room (about 20°C) under a 12/12-h light/dark cycle (lights on from 1800 to 0600 h). During the cone field

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measurements, rats were subjected to a food-deprivation regimen, in which 6 g dry laboratory chow were given at the end of each day of testing. When not tested, i.e., during weekends, rats had free access to food for 1 day. The rats were not housed in the same room in which they were tested. A radio, which was playing softly, provided background noise in all rooms. All testings were done between 0900 and, maximally, 1800 h.

2.2. Treatment

Metrifonate was freshly dissolved in 0.1 M sodium citrate buffer (pH 5.5) on each experimental day. Rats were randomly assigned to four dosage groups (vehicle, low dose [3 mg/kg], medium dose [10 mg/kg] and high dose [30 mg/kg] metrifonate). Drug or vehicle was given p.o. (injection volume, 1 ml/kg) 30 min before testing. Each metrifonate-treated group consisted of seven animals, while the control (citrate-treated) group consisted of 14 animals. The control group was also used for another drug testing study.

2.3. Spatial cone field task

The cone field apparatus, which is fully automated, has been described in detail elsewhere (van der Staay et al., 1990b). The experimental room was illuminated by red fluorescent strip lights and one light bulb, which were adjusted by a dimmer to give an intensity of about 50 lx on the floor of the apparatus. Extra-maze cues consisted of one door, a window (covered by a black curtain), a sink, and two tables, on one of which was a computer and interface. The experimenter sat in front of the apparatus and was present and visible throughout the behavioral testing.

The rats were familiarized with the cone field through 10-min trials on consecutive days. All cones contained one 45-mg food pellet (Noyes) during the adaptation trials. Rats were also adapted to the p.o. injections by injecting them with vehicle 30 min before each adaptation trial. As soon as a rat reached criterion performance, i.e., it collected nine or more food pellets in one adaptation trial, training started. Now only four cones of a fixed set contained one food pellet each. To avoid possible odor cues, all cones were provided with a reservoir on top of the cone that contained six pellets. The rats could not get these pellets. A trial was started by placing the rat in the start box. Within a series of daily trials, the starting position was determined by random permutations of the numbers, 1–4. The sliding door was then opened immediately. As soon as the rat had entered the cone field, the sliding door was closed. Whenever the rat touched the top of a cone, it closed an electrical circuit and activated a counter. This was scored automatically as a cone visit. Only contacts preceded by a visit to another cone were scored (excepting the first cone visit). Infrared photocells detected whether a rat poked its nose onto the top of the cone. A personal computer collected the data and controlled the duration of the trials. A trial was terminated when the rat had found and consumed all four food pellets or when 7 min had elapsed, whichever occurred first. The animal was put back into its home cage between trials. After the cone field had been cleaned with a damp sponge, and the four cones had been rebaited, the next trial was started. As many trials as possible were given within 15 min. This means that on the first days of training, only two trials were given daily. A maximum number of six trials per day was given. Trials in which no or only one food pellet was collected were regarded as incomplete (van der Staay et al., 1990b). Because this type of trial induces a strong bias with respect to the performance measures, the data for incomplete trials were excluded from analysis. Training was continued until 60 correct trials had been achieved.

2.4. Statistical analysis

Several variables were used (e.g., first choice latency, number of rewarded visits, etc.) to calculate the following measures.

2.4.1. Working memory

This refers to the number of rewarded visits per number of visits to the baited set of cones. This ratio reflects the ability of rats to avoid revisits to baited cones during a trial.

2.4.2. Reference memory

This refers to the number of visits to the baited set of cones per number of visits to all cones. This ratio provides an index of the ability of the rats to discriminate between baited and non-baited cones. Working and reference memory were assumed to be indices of spatial discrimination performance in the cone field task (van der Staay et al., 1990b). If a rat makes four visits and collects all rewards, both measures have the value 1 (optimal/perfect performance).

2.4.3. Choice correspondence of reinforced visits

The choice correspondence was calculated pair-wise between two successive trials (i.e., 1–2, 2–3, 3–4, etc.). The longest common sequence of reinforced visits in these two trials was taken as the measure of correspondence. Note that only visits to baited cones were used for this analysis. This measure, which could range from 1 to 4, gives an impression of the search pattern of rats. If rats visit the rewarded cones in a random order from trial to trial, this measure will approximate the value 1.72 (van der Staay, 1999; van der Staay et al., 1990b). A value of 4 would indicate that the rats visited the baited cones in the same manner on consecutive trials, i.e., they had developed a fixed food search pattern.

2.4.4. Intervisit interval time

This refers to the time between the first and last visits in a trial per number of visits minus one. This measure provides a mean value of the choice latencies between cone visits (baited and non-baited).

The means for the recorded and calculated measures were averaged for blocks of 10 trials. Data from trial blocks 2–6 only were analyzed because trials were often incomplete at the start of training, which would confound the results (van der Staay et al., 1989). Dose effects of metrifonate were analyzed using a two-factorial (Dose × Block) analysis of variance with Block as repeated measures. Post-hoc analyses of differences between doses were performed using a Duncan's post-hoc multiple range test (using a 0.05 confidence interval).

Two rats were removed from the experiment because they did not reach the criterion to start training, even after 19 adaptation trials. There were thus 13 rats in the control group, and six in the group treated with 10 mg/kg metrifonate.

3. Results

At the start and end of testing, the rats weighed 539 ± 9 g and 485 ± 9 g (means ± S.E.M.), respectively. The mean number of adaptation trials (one per day) to reach criterion performance was not different between groups and was 8.4 ± 0.6 (means ± S.E.M.). Thus, there were no behavioral differences between the experimental groups before training and treatment started. The number of days taken to complete 60 training trials (maximally six trials per day) did not differ between the four groups and was 20.3 ± 0.5 (mean ± S.E.M.).

The effects of metrifonate on the main measures in the cone field task are shown in Fig. 1. All animals improved their working and reference memory performance during training, indicating that the rats learned the spatial cone field task (Block effect: both $F > 15.13$, $P < 0.01$; see Fig. 1A and B). When averaged over the last five trial blocks, metrifonate treatment was found to improve the working memory performance (general mean Dose effect: $F(3,29) = 3.04$, $P < 0.05$). Post-hoc analysis on the general mean of the last five trial blocks revealed that only the highest dose metrifonate improved the average working memory performance when compared to the control group. Examining treatment effects on individual trial blocks, an effect on working memory performance was found only for the sixth (last) trial block (Dose effect: $F(3,29) = 4.10$, $P < 0.05$). The post-hoc test revealed that rats treated with 30 mg/kg metrifonate had a better performance than the rats treated with vehicle or 3 mg/kg metrifonate. Metrifonate had no effect on reference memory performance (see Fig. 1B).

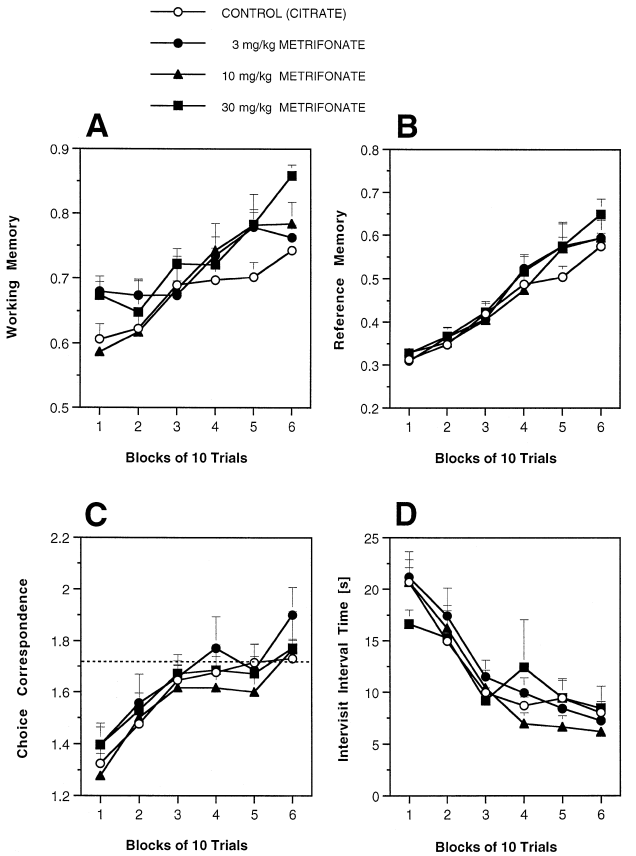

Fig. 1. Effects of metrifonate (3, 10 and 30 mg/kg, p.o.) on performance in the spatial cone field task. (A) Working memory; (B) reference memory; (C) choice correspondence of reinforced visits; and (D) intervisit interval.

In the course of training, the choice correspondence, i.e., the order of reinforced visits from trial to trial, reached the chance level, i.e., 1.72 (Block effect: $F(5,116) = 4.57$, $P < 0.01$; see Fig. 1C), and the intervisit interval time, i.e., the time between cone visits, decreased (Block effect: $F(5,116) = 25.67$, $P < 0.01$; see Fig. 1D). Metrifonate treatment had no effect on these and other measures of timing, e.g., first choice latency (latency of first cone visit).

4. Discussion

We found that the highest dose of metrifonate (30 mg/kg) improved the working memory performance of middle-aged rats in the spatial cone field task, whereas it had no effect on reference memory performance. The rats had no preferred pattern of visiting the baited cones, i.e., they did not have a fixed food search pattern. Therefore, it can be assumed that the rats used a spatial orientation strategy. Furthermore, because metrifonate treatment did not affect activity measures, the improvement in spatial working memory appears to be a specific effect on spatial

memory. The observation that metrifonate improved working memory but not reference memory performance supports the view that working and reference memory do not represent the same aspect of spatial memory as assessed in the cone field task (van der Staay et al., 1990a). As the cone field task allows the simultaneous assessment of both working and reference memory, this test is considered particularly suited to identify the effects of drugs on different aspects of spatial memory.

Metrifonate has previously been found to improve memory performance, especially in the early phase of spatial orientation learning of rats in the Morris water escape task (van der Staay et al., 1996). The Morris task is generally considered to measure spatial reference memory performance, because the animal has to acquire the location of a fixed escape platform which is constant across sessions. Since we did not find any effects of metrifonate on the reference memory performance in the cone field task, these results may appear contradictory. However, previous studies have shown that the performance on measures with the same behavioral definitions (e.g., reference memory) in different spatial tasks are not correlated (van Luijtelaar et al., 1989; Blokland and Raaijmakers, 1993). This indicates that different aspects of behavior are involved in the performance (e.g., reference memory) in different tests. Consequently, this may indicate that different aspects of behavior are affected by metrifonate treatment, leading to different results in different tests.

In a radial maze task, in which all arms were baited, metrifonate was found to improve performance, i.e., it was found that the treated rats made more correct choices until a first revisit (Dachir et al., 1997). This measure can be regarded as an index of working memory (Olton et al., 1980). Thus, this finding corroborates our data, suggesting that metrifonate improves working memory performance. In the Morris task, metrifonate was found to improve the performance already during the first four acquisition trials, which were given in close succession (van der Staay et al., 1996). This effect might also be indicative for an improvement in working memory performance. However, during the first trials of training in the Morris task, the rats experienced stress and had to learn the procedures of the task. Consequently, the improved performance after metrifonate treatment in the Morris task could also be explained by effects of the treatment on these aspects of behavior.

Repeated oral administration of metrifonate at a dose of 30 mg/kg body weight, i.e., the highest dose tested in this study, causes, at best, a very weak inhibition of acetylcholinesterase in the rat brain ($\leq$ 20%; Hinz et al., 1998). Thus, the cognition-enhancing effect seen in this study cannot fully be explained by acetylcholinesterase inhibition, as has been suggested earlier (van der Staay et al., 1996; Itoh et al., 1997). Since the cognition-enhancing effect was seen only with the highest dose used, i.e., 30 mg/kg, it might be interesting to test higher doses of metrifonate which are able to induce clinically relevant inhibition of acetylcholinesterase. However, these higher doses are associated with the first signs of adverse effects of cholinergic overstimulation, effects which are not yet seen at the dose of 30 mg/kg (Blokland et al., 1995; Hinz et al., 1996). Therefore, a dose of 30 mg/kg metrifonate is appropriate to investigate the cognition-enhancing effect of metrifonate.

Taken together, the results of the present study indicate that metrifonate selectively improves the working memory performance of middle-aged Wistar rats in the cone field task without affecting reference memory performance, the activity level, or the strategy applied to negotiate the cone field.

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