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Cholinergic drugs affect novel object recognition in rats: Relation with hippocampal EEG?

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Abstract

This study examined the role of cognitively enhancing cholinergic drugs on both object memory and brain activity in rats, as well as the possible relation between the two measures. A group of twenty-four animals was used for assessing object recognition. In another group of eight rats, an electrode was implanted into the dorsal hippocampus to record an electroencephalogram (EEG) and auditory evoked potentials (AEP). In both groups, animals were treated with saline, 0.1 mg/kg scopolamine, 0.1 mg/kg methylscopolamine, 3 mg/kg donepezil, donepezil combined with scopolamine, 0.1 mg/kg nicotine, and nicotine combined with scopolamine. Scopolamine, but not methylscopolamine, impaired object recognition. Both donepezil and nicotine reversed this impairment. The N1 and N2 components of the AEP became closer to baseline after scopolamine, which was not reversed by donepezil or nicotine. Scopolamine increased the theta frequency in the EEG. When combined with donepezil, theta increased even more. Conversely, nicotine reversed the theta increment to control level. It is suggested that scopolamine caused a decrement in arousal in this study. Furthermore, the current results suggest a relation between EEG and object memory after cholinergic drug treatment. However, there was a clear dissociation between memory performance and EEG after combined treatment with drugs, which makes additional research where EEG and performance measures are co-registered imperative.

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Keywords: Acetylcholine; Animal model; Auditory evoked potential (AEP); Donepezil; Electroencephalogram (EEG); Nicotine; Scopolamine

1. Introduction

Previous studies have shown that the object recognition task in rats is highly sensitive to psychoactive drug treatments. Various drugs such as acetylcholinesterase inhibitors can improve object memory performance in these animals (Lieben et al., 2005; Prickaerts et al., 2005). This suggests that such drugs may also be useful in treating memory impairments in humans. However, clear evidence for a reliable memory enhancing effect of these drugs in humans is lacking, which is possibly related to the discrepancy between the large numbers of animal studies showing memory improvement after treatment

with acetylcholinesterase inhibitors, whereas there are only a limited number of human studies showing memory enhancing effects of drugs. Studies using rats as subjects show that acetylcholinesterase inhibitors such as metrifonate, rivastigmine, and donepezil can reverse drug-induced memory impairments (Bejar et al., 1999; Lieben et al., 2005; van der Staay and Bouger, 2005; Yamada et al., 2005). However, only Snyder and colleagues (Snyder et al., 2005) were able to improve a scopolamine-induced memory impairment in healthy elderly volunteers using donepezil, whereas a single dose of rivastigmine even impaired recognition memory in the study of Wezenberg et al. (Wezenberg et al., 2005). Furthermore, Mumenthaler and colleagues showed that nicotine and donepezil mainly improved sustained attention processes rather than learning and memory (Mumenthaler et al., 2003). It must be noted, however, that acetylcholinesterase inhibitors may improve memory to some extent in clinical populations (e.g., Birks, 2006; Yoo et al., in press).

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EEG characteristics show great similarities across species. For example, alertness is associated with fast frequencies in the EEG (e.g., beta activity), whereas non-REM sleep and drowsiness are characterized by slower waves (synchronized firing of cortical neurons). Also, evoked potentials (EPs) show great correspondence between rats and man (Sambeth et al., 2003, 2004; Sambeth and Maes, 2006). In both species, the short latency EP components are related to the processing of the physical properties of a stimulus, whereas the later components are associated with more endogenous processing, e.g., the psychological processes involved in the stimulus event (e.g., Sambeth et al., 2003; Sambeth and Maes, 2006). Therefore, drug-induced changes in EEG characteristics in animals may be used to predict central activity of drugs in humans. Clearly, such an approach can also be used if first a relation between EEG and memory performance can be found in animals.

Previous work has shown that drugs affect EEG characteristics in humans and rodents in a similar manner (Coenen and Van Luitelaar, 2003; Dimpfel, 2005; Dimpfel et al., 1992; Jongsma et al., 1998a,b). However, whether drug effects on memory performance are related with EEG characteristics in rats has not been tested yet. Therefore, we evaluated drug effects in an object recognition task (memory) and examined the effects of the same drugs on hippocampal auditory evoked potentials (AEP) and theta activity in two groups of rats. Auditory stimuli were chosen, since they reveal a strong correspondence between rats and humans (Sambeth and Maes, 2006; Sambeth et al., 2004). Furthermore, Campbell and co-workers (Campbell et al., 1995) have suggested that a disruption of muscarinic cholinergic receptor activation in the extralemnic auditory pathways will lead to changes in AEP components (in this study, acetylcholine was manipulated).

We used scopolamine to induce a memory impairment using an object recognition task. Methyl-scopolamine, which only differs from scopolamine in that it does not cross the blood-brain barrier, was used as a control. Both donepezil, an acetylcholinesterase inhibitor, thereby preserving synaptic and presynaptic acetylcholine, and nicotine, a nicotinic receptor agonist, were administered alone or together with scopolamine in order to examine whether they were able to reverse both the scopolamine-induced memory impairments and the scopolamine effects on the AEP and the EEG. The EEG was recorded from the dorsal hippocampus, since previous work has shown this brain area to be involved in object recognition (Broadbent et al., 2004; Clark et al., 2000; Prickaerts et al., 2002a).

2. Materials and methods

2.1. Animals

The study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethical committee of the Maastricht University for animal experiments was obtained. Eight (for EEG study) and 24 (for memory study) 3-month-old male Wistar rats (Harlan, Horst, The Netherlands) served as subjects. The animals were singly housed in standard Makrolon

cages on sawdust bedding in an air-conditioned room (around 21 °C). They were maintained on a 12–12 h light–dark cycle with lights off at 5.00 a.m. and had unlimited access to food and water. The rats also had a block of wood in their cages to sit on or to gnaw on. Rats were housed in the same room as where they were tested. A radio, which was playing softly, provided background noise.

2.2. Surgical procedures and recordings of EEG study

Animals were anesthetized with ketamine 50 mg/kg i.m. and additionally received an s.c. injection of the muscle relaxant xylazine 4 mg/kg. Next, they were placed in a stereotaxic frame. After exposing the skull, the animals received several drops of the local anesthetic lidocaine before the bregma intersection was identified. One active lead in the dorsal hippocampus (anterior–posterior -3.2 , medial–lateral 1.6 , dorsal–ventral -2.6) was inserted, related to bregma. It was referenced to the cerebellum. The ground was also placed in the cerebellum. Three screws and dental acrylic cement were employed to fix the electrode on the skull surface. After the surgery, the animals received an injection of Temgesic (0.1 mg/kg, s.c.) to reduce pain. Next, they were allowed to recover for at least two weeks.

Field potentials (electroencephalogram, EEG) were filtered between 1 and 133 Hz and sampled at 1000 Hz. The results were stored on a personal computer and analyzed using Neuroscan software.

Each session started with the injection of the drugs. Thirty minutes later, rats were attached to the EEG equipment and EEG was visually checked. This was followed by the presentation of 120 auditory stimuli. They had a duration of 500 ms, a frequency of 2500 Hz and an intensity of 80 dB. The inter-stimulus interval was 2000–4000 ms.

2.3. Object recognition memory and procedure of memory study

The object recognition test was performed as described elsewhere (e.g., Prickaerts et al., 2005, 2002b). The apparatus consisted of a circular arena, 83 cm in diameter. Half of the 40 cm high wall was attached with a black and white plastic topping, the other half of transparent polyvinyl chloride. Fluorescent red tubes and a light bulb provided a constant illumination of about 20 lux on the floor of the apparatus. The light intensity was equal in the different parts of the apparatus. Two objects were placed in a symmetrical position about 10 cm away from the wall. Each object was available in triplicate. Eight different sets of objects were used. The different objects were; 1) a cone consisting of a gray polyvinyl chloride base (maximal diameter 18 cm) with a collar on top made of brass (total height 16 cm), 2) a bottle of brown glass (diameter 10 cm, height 22 cm) filled with water, 3) a massive metal cube (10.0×5.0×7.5 cm) with two holes (diameter 1.9 cm), 4) a massive aluminum cube with a tapering top (13.0×8.0×8.0 cm), 5) a stoneware flowerpot with a 1 cm opening on top (maximal diameter 12.0 cm), 6) a round varnished vase with a slender neck (maximal diameter round part 9.0 cm, height 13.0 cm, neck 3.5 cm, and height

5.0 cm), 7) a red can with relief (12.0×9.0×9.0 cm), and 8) a round white cup with a handle (diameter 7.0 cm and height 9.5 cm). A rat could not displace the objects.

A testing session started with the injection of the drugs. Thirty minutes later, a rat was subjected to the first trial (T1) which lasted 3 min. During T1 the apparatus contained two identical objects (samples). A rat was always placed in the apparatus facing the wall at the middle of the transparent segment. After the end of T1 the rat was put back in its home cage. After one hour the rat was put back in the apparatus for the second trial (T2), but now with two dissimilar objects, a familiar one (the sample) and a new one. The times spent in exploring each object during T1 and T2 were recorded manually with a personal computer.

Exploration was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered as exploratory behavior. In order to avoid the presence of olfactory trails the objects were always thoroughly cleaned. Also, there were three copies of each object. Since the familiar copy was not used in T1, the rat could not use olfactory cues to recognize the familiar object. All combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects.

In several studies it was shown that Wistar rats show a good object memory performance when a one-hour delay is interposed between the first and the second trial. However, when a 24 h delay is used the rats do not discriminate the novel and the familiar in the second trial, indicating that the rats do not remember the object that was presented in the first trial (e.g., Lieben et al., 2005; Prickaerts et al., 2005). Using a six hour delay, the discrimination performance is between the performance of the one hour and 24 h delay, suggesting a delay-dependent forgetting in this task.

2.4. Drug treatment

Scopolamine hydrobromide, methyl-scopolamine, and nicotine were obtained from Sigma (Zwijndrecht, The Netherlands). Donepezil was received from Apin chemicals (Oxon, United Kingdom). Scopolamine (0.1 mg/kg), methyl-scopolamine (0.1 mg/kg), and nicotine (0.1 mg/kg) were freshly dissolved in saline and injected i.p. in a volume of 2 ml/kg. Saline was also injected in the same volume i.p. Donepezil (3 mg/kg) was dissolved in MilliQ pure water and administered p.o. in a volume of 1 ml/kg. Saline, scopolamine, methyl-scopolamine, and donepezil were administered 30 min before T1 and nicotine 20 min before T1.

Scopolamine, methyl-scopolamine, and saline treatments were performed twice. The results of those two measurements were later averaged.

2.5. Analyses

2.5.1. EEG analysis

First, EEG was visually checked offline for artifacts. AEPs generated by stimuli that were presented in the presence of

artifacts were excluded from further analysis. The EEG fragments within an epoch of 100 ms before onset of a response and 1000 ms after response onset were averaged for all trials without artifacts. The mean amplitude of the 100 ms before stimulus onset was used as a baseline value (baseline correction).

The AEP components were determined on the basis of the individual (single animal) and grand average (over all animals) AEPs, taking both the latency and amplitude variables into account. The amplitude of the P1 component was defined as the maximum positive amplitude between 20 and 42 ms after stimulus onset, N1 as most negative value between 37 and 55 ms after tone onset, P2 as most positive between 60 and 80, and N2 as most negative between 160 and 220 ms.

Analyses of variance (ANOVA) was used for the analyses of each AEP component. Three main analyses were performed. First, the saline AEPs were compared to the scopolamine and methyl-scopolamine AEPs, using Treatment (3 levels) as within-subject factor. The second analysis comprised ANOVAs for the comparison of scopolamine with donepezil (4 levels: saline, scopolamine, donepezil, scopolamine+donepezil). In the final analysis, scopolamine was compared to nicotine (4 levels: saline, scopolamine, nicotine, scopolamine+nicotine). Post hoc analyses using least significant difference tests were performed to evaluate differences between conditions in more detail ($P < .05$).

Next, we performed a frequency analysis on the data. After baseline correction was performed on the data (using the last 100 ms before stimulus onset), the EEG fragments from onset of the tone until 1000 ms after tone onset were included in a spectral analysis. The spectral content of the EEG was determined by Fast Fourier Transform (FFT, frequency resolution of 1 Hz), with a Hamming correction. For each tone, the FFT was calculated. An average over stimuli was subsequently calculated. Only the absolute power of the theta frequency band (5–10 Hz) was used for statistical analysis. ANOVA's were calculated in a similar manner as the analyses for the AEP components.

2.5.2. Behavioral analysis

The basic measures were the times spent by rats in exploring an object during T1 and T2. The time spent in exploring the two identical samples will be represented by 'a1' and 'a2'. The time spent in T2 in exploring the sample and new object will be represented by 'a' and 'b', respectively. The following variables were calculated: 1) Total exploration during T1 ($e1$)= $a1+a2$, 2) total exploration during T2 ($e2$)= $a+b$ and 3) Discrimination score ($d2$)= $b-a/e2$. The $d2$ was considered as an index measure of discrimination between the new and the familiar objects. In fact, $d2$ is a relative measure of discrimination that corrects for exploration activity ($e2$). Thus, there should be no differences in $d2$ indices between experiments with similar treatments at similar intervals.

Effects between the different drug conditions were assessed using a one-way ANOVA. In case of a statistically reliable dose effect, comparisons between means of the different doses were analysed in more detail using post hoc least significant difference tests ($P < .05$).

3. Results

3.1. Missing data

Two animals were not used for the analysis, since the EEG was generally of too bad quality due to technical problems. Postmortem verification of the hippocampal electrode was performed in the other six animals. The brains were removed and coronal sections were cut. Next, they were stained using hematoxyline and eosine and inspected under a light microscope. In four out of six animals, the electrode was located in the dorsal hippocampus. In the other two, no electrode position could be located at all. However, the recordings of these two animals showed a reliable EEG signal with characteristic hippocampal EEG, comparable to the other rats.

3.2. AEP components

3.2.1. Saline vs. scopolamine treatment

Fig. 1A shows the hippocampal AEPs after saline, scopolamine and methyl-scopolamine treatment. Four main components are present: P1, N1, P2, and N2. It can be noted that scopolamine decreases most of the amplitudes of the AEP components.

The ANOVAs did not show any significant effects with regard to the amplitudes of the AEP components. The N1, however, revealed a marginally significant effect of treatment ($F_{2,10}=3.58$, $P<0.07$). The amplitude of the N1 was significantly increased after scopolamine compared to the saline condition ($P=0.03$), as shown by the post-hoc analysis. Furthermore, the N2 revealed a marginally significant treatment effect ($F_{2,10}=3.57$, $P<0.07$), which was indicated by a significant decrease in amplitude after scopolamine than after saline ($P=0.02$).

The latencies of the components were not affected by drug treatment.

3.2.2. Donepezil treatment

Fig. 1B shows the AEPs after the saline, scopolamine, donepezil and scopolamine+donepezil treatment conditions. Donepezil treatment did not reverse scopolamine-induced changes in the amplitudes of the AEP components.

The P1 component was not affected by donepezil treatment. Treatment did significantly affect the N1 ($F_{3,15}=8.04$, $P<0.01$). Post-hoc analysis revealed that the N1 was larger after scopolamine and after scopolamine+donepezil treatment as compared with saline. Furthermore, the scopolamine+donepezil amplitude was larger than the donepezil amplitude.

The P2 component of the AEP was not affected by treatment. Treatment did have an effect on the amplitude of the N2 ($F_{3,15}=8.17$, $P<0.01$). Post-hoc analysis showed that the scopolamine N2 amplitude was decreased compared to the saline and donepezil treatment. Furthermore, N2 was larger to the donepezil than to the scopolamine+donepezil component.

The ANOVAs for the latencies of the components revealed no drug effects.

3.2.3. Nicotine treatment

The AEPs after saline, scopolamine, nicotine and scopolamine+nicotine treatment are shown in Fig. 1C. As was the case for the donepezil treatment, nicotine treatment was not able to reverse the scopolamine-induced effects.

The ANOVA for the AEP N2 component was the only component to show a significant amplitude effect ($F_{3,15}=3.38$, $P<0.05$). Post-hoc analysis revealed that scopolamine elicited smaller N2 amplitudes than did saline and nicotine treatments.

None of the latencies of the AEP were affected.

Fig. 1. Hippocampal grand average AEPs after scopolamine (1A), donepezil (1B), and nicotine (1C) treatments. Latencies are shown on the x-axis in milliseconds and amplitudes are presented on the y-axis in microvolts. Note that four AEP components were present, which are nominated P1, N1, P2, and N2.

Fig. 2. Absolute theta power (and standard error) in response to auditory stimuli after scopolamine (2A), donepezil (2B), and nicotine (2C) treatments. The power is represented as square microvolts (μV^2).

3.3. Frequency analyses

3.3.1. Saline vs. scopolamine treatment

Fig. 2A shows the absolute theta power during auditory stimuli, for the scopolamine treatment. The ANOVA revealed a significant treatment effect ($F_{2,10}=6.16$, $P<0.03$). Post-hoc analysis showed that theta power was larger after scopolamine than after methyl-scopolamine treatment.

3.3.2. Donepezil treatment

Theta power after donepezil treatment can be seen in Fig. 2B. It was significantly affected by treatment ($F_{3,15}=7.46$, $P<0.01$). Post-hoc tests revealed that combined scopolamine and donepezil treatment elicited larger theta power than did the other three treatment conditions. There were no differences between saline, scopolamine, and donepezil.

Fig. 3. Discrimination (D2) scores (and standard error) from the second trial of the object recognition task, for scopolamine (3A), donepezil (3B), and nicotine (3C) treatments.

3.3.3. Nicotine treatment

Fig. 2C illustrates the theta power after nicotine treatment. The ANOVA showed a significant treatment effect ($F_{3,15}=6.58$, $P<0.01$). Theta power was decreased after nicotine treatment compared to saline and scopolamine, as shown by the post-hoc test. Furthermore, combined scopolamine and nicotine theta power was decreased compared to scopolamine alone.

3.4. Object recognition task

3.4.1. Saline vs. scopolamine treatment

e1 during the first trial was significantly affected by treatment ($F_{2,47}=5.42$, $P<0.01$). Post-hoc analysis showed that exploration was larger after saline than after the other two treatments. Exploration during Trial 2 did not differ between treatments ($F_{2,47}=0.34$, n.s.).

Fig. 3A shows the *d2* score of the animals in the second trial of the object recognition task after saline, scopolamine, and methyl–scopolamine. The ANOVA revealed a significant treatment effect ($F_{2,47}=11.78$, $P<0.001$). The *d2* score was decreased in the scopolamine condition compared to both the saline and the methyl–scopolamine conditions, as shown by the post-hoc analysis. Saline and methyl–scopolamine *d2* scores did not differ.

3.4.2. Donepezil treatment

Treatment did not significantly affect *e1* ($F_{3,70}=2.51$, n.s.) and *e2* ($F_{3,70}=1.36$, n.s.) exploration scores.

Fig. 3B illustrates the *d2* score after donepezil treatments. A significant treatment effect was found ($F_{3,70}=10.21$, $P<0.001$). This was due to decreased *d2* scores in the scopolamine condition compared to the other three treatments. Donepezil, thus, reversed the decrement in *d2* score induced by scopolamine.

3.4.3. Nicotine treatment

A significant treatment effect was found with regard to *e1* ($F_{3,57}=2.84$, $P<0.05$). Exploration was larger after saline than after scopolamine and nicotine treatments, but saline and nicotine+scopolamine did not differ in exploration time, as shown by a post-hoc analysis. Exploration during the second trial (*e2*) did not significantly differ ($F_{3,57}=0.65$, n.s.).

d2 scores after nicotine treatments can be seen in Fig. 3C. A significant treatment effect was present ($F_{3,57}=12.86$, $P<0.001$). Post-hoc analysis revealed that the *d2* score was smaller after scopolamine compared to the other treatments, which indicated that nicotine reversed the scopolamine induced *d2* decrement.

4. Discussion

The aim of this study was to assess whether drug effects on memory can be related to drug effects on EEG in rats. We examined the effects of scopolamine, methyl–scopolamine, donepezil, and nicotine on object recognition and AEPs in two groups of animals. Object recognition was impaired after treatment with scopolamine, but not after methyl–scopolamine.

Both donepezil and nicotine were able to reverse this impairment. With regard to the AEPs, scopolamine increased the amplitude of the N1 component, but decreased the N2 component amplitude. Neither donepezil nor nicotine was able to reverse these AEP effects. Scopolamine increased theta frequency. When combined with donepezil, theta was even more increased. Nicotine, on the other hand, was able to reverse this theta increment, as was shown by the decrement in theta when scopolamine and nicotine were combined as compared to the scopolamine treatment alone.

Not unexpectedly, the current findings that scopolamine but not methyl–scopolamine impaired object recognition, are in accordance with previous research in animals (Blokland, 1995; Cosquer et al., 2005; Dodart et al., 1997; Woolley et al., 2003) and humans (Ebert and Kirch, 1998; Schon et al., 2005; Sherman et al., 2003).

In our study, scopolamine increased the theta frequency 30 min after treatment as compared to the methyl–scopolamine treatment. This corresponds to most animal (Dimpfel, 2005; Masuoka et al., 2006) and human (Ebert et al., 1998; Sannita et al., 1987; Sloan et al., 1992) data. Only Kinney and colleagues (1999) showed a decrement in theta after scopolamine in urethane-anesthetized animals, possibly because the anesthesia interacted with the scopolamine.

Hippocampal theta is related to motor activation or active sniffing behavior in rats and may lead to the formation of memories (Buzsaki, 2005; Vanderwolf, 1969, 1992). Theta frequency in humans, which is usually measured from the scalp, may however be related to both specific types of activation (such as seen during concentration in a chess-game) or drowsiness (e.g., Smit et al., 2004; Burgess and Gruzelier, 2000; Klimesch et al., 2000). It has been suggested that theta increments are due to a decline of information processing (Smit, 2004; Klimesch et al., 2000). In the case of a vigilance decrement, information processing in general is diminished due to lowered arousal, whereas only irrelevant information processing is diminished in the case of specific activation. Both situations may lead to increments in theta activity, which may make it difficult to compare rat data to those in humans. Considering that changes in theta frequency in animals are due to the same processes as proposed in humans, the increment in theta activity after scopolamine treatment in the present study may be due to 1) a decline of information processing other than the auditory stimuli presented and 2) a general arousal decrement after scopolamine.

At the dose used, methyl–scopolamine did not change theta frequency in the current experiment. To our knowledge, the effect of methyl–scopolamine on EEG frequencies has not been studied so far in rats. On the other hand, since methyl–scopolamine crosses the blood-brain barrier only to a limited extent (Moore et al., 1992), such a lack of an effect could be expected. One study in humans (Sloan et al., 1992) showed that methyl–scopolamine did not affect EEG activity, a result comparable to ours.

Next to the effects on the EEG, we examined the effects of scopolamine on the rat AEP. Scopolamine increased the N1, but decreased the N2 component. A closer look at the AEPs (see

Fig. 1), however, reveals that the amplitudes of both components became closer to baseline (0 mV) after scopolamine. This is similar to an effect previously found after repeated auditory stimulation in rats (Sambeth and Maes, 2006; Sambeth et al., 2004). Thus, it may be concluded that both components decreased in amplitude after scopolamine treatment in our study (see also Campbell et al., 1995). In our study, methyl-scopolamine did not affect the AEP components, as was found in the study of Campbell and colleagues as well (Campbell et al., 1995).

In sum, scopolamine, but not methyl-scopolamine, impaired object recognition, reduced the amplitudes of the N1 and N2 components, and increased theta activity. These data underscore the notion that the changes after scopolamine are due to its central action in the brain, rather than an effect on the peripheral muscarinic receptors.

One possible explanation of these results might be that they are due to a drop in arousal after scopolamine. Scopolamine is known to decrease vigilance and impair attention processes in humans (Ellis et al., 2006; Kamboj and Curran, 2006; Koller et al., 2003; Mintzer and Griffiths, 2003). The impairments in object recognition and the decrease in N1 and N2 amplitudes found in the current study could be explained by those arousal processes. Furthermore, theta frequency was increased in the hippocampus, which can be a result of the arousal decrement as well.

Even though some evidence is in favor of a decrement in arousal in this experiment, other studies in which acetylcholine was manipulated using scopolamine have argued against this possibility (Curran et al., 1998; Molchan et al., 1992; Sloan et al., 1992). For instance, scopolamine did not significantly affect the latencies of several visual evoked potential components in humans although they are susceptible to drowsiness effects (Sloan et al., 1992). Furthermore, sedative effects of scopolamine are often independent from effects on memory (Curran et al., 1998; Molchan et al., 1992). Future research, in which effects on memory, attention, and arousal will have to be directly compared, will shed more light into the specificity of the effects found in the present study.

In this experiment, the drugs that increased acetylcholine were able to reverse the scopolamine-induced object recognition impairment, which is in line with other work showing that donepezil and nicotine can improve memory performance in rats (Barros et al., 2005; Prickaerts et al., 2005; Yamada et al., 2005). Thus, this again shows that both the muscarinic and the nicotinic cholinergic receptors play a role in memory processing (for reviews, see Gold, 2003; Levin et al., 2006; Parent and Baxter, 2004). Furthermore, the fact that nicotine reversed the scopolamine-induced impairment suggests that the nicotinic and muscarinic receptors functionally interact in their effect on memory (see also Green et al., 2005).

It must be noted that high doses of scopolamine may not only have an effect on the muscarinic receptors, but also on the nicotinic receptors (Schmeller et al., 1995). In the current experiment, we only used a relatively low dose of 0.1 mg/kg as opposed to other studies where doses of between 0.25 and 1 mg/kg have been administered (Dimpfel, 2005; van der Staay

and Bouger, 2005; Bejar et al., 1999; Kinney et al., 1999; Campbell et al., 1995). It is therefore, not likely that nicotine reversed the scopolamine-induced impairments because of scopolamine's effect on the nicotinic receptor.

Neither donepezil, nor nicotine, was able to reverse the effects of scopolamine on the N1 and N2 components of the AEP. These findings suggest that the drug effects on the AEP and the memory performance are independent and argue against a relation between these two parameters. Thus, although scopolamine affects both the AEP and the object memory performance, a dissociation between these effects was found after treatment with donepezil and nicotine.

Donepezil did not reverse the scopolamine-induced increase in hippocampal theta frequency, whereas nicotine did. Actually, donepezil combined with scopolamine resulted in an even larger increment of theta compared to scopolamine treatment alone. The fact that both scopolamine (Dimpfel, 2005; Masuoka et al., 2006) and donepezil (Kinney et al., 1999) increase theta activity when given separately might explain this current effect. However, in the study of Kinney et al. (Kinney et al., 1999) theta increment after donepezil was reversed by scopolamine. As mentioned earlier, the discrepancy between the data is likely to be explained by the urethane-anesthesia used in the study by Kinney and colleagues.

Above, it was proposed that scopolamine decreased arousal level, which in turn increased theta activity. If this is indeed the case, then the effect on theta activity by nicotine shows that it is able to increase arousal. After all, theta frequency did not differ between nicotine and the scopolamine-nicotine combination, and, was smaller than after scopolamine alone. However, a stabilization of the arousal level due to nicotine should have increased the N1 and N2 components, which it did not do. Therefore, nicotine was only partly able to reverse the scopolamine-induced arousal decrement. Given the effects of donepezil on the AEP components and theta frequency in the current experiment, we would conclude that donepezil did not reverse a scopolamine-induced decrement in arousal at all.

An explanation for the difference in effects after donepezil and nicotine treatment may come from the fact that nicotine binds to nicotinic acetylcholine receptors, which may lead to an increase in dopamine levels in the brain (Turner, 2004; Wonnacott, 1997; Wang et al., 2007). Donepezil only inhibits the acetylcholinesterase (for review see Seltzer, 2007), leading to enhanced acetylcholine levels in general. Possibly, arousal levels were partly increased after nicotine treatment because of an increase in dopamine rather than acetylcholine in general, and thus, the nicotinic receptors may be more important than muscarinic receptors with respect to arousal. Further research is needed to test this hypothesis.

In conclusion, scopolamine impaired object recognition and affected hippocampal EEG (AEP and theta power). Although these data alone may suggest a relation between memory performance and EEG, the findings of the additional drugs donepezil and nicotine caution such a simple conclusion. There was a clear dissociation between memory performance and EEG after combined treatment with drugs. Apparently, drug effects on behavior and EEG are dissociable on basis of

different processes. Further studies are indicated in which task-related EEG measures are associated with memory performance. Such studies may further explore the possibility to use EEG measures for translational studies.

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