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ORIGINAL INVESTIGATION

Mnemonic and behavioral effects of biperiden, an M1-selective antagonist, in the rat

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Abstract

Rationale There is a persistent pressing need for valid animal models of cognitive and mnemonic disruptions (such as seen in Alzheimer's disease and other dementias) usable for preclinical research.

Objectives We have set out to test the validity of administration of biperiden, an M1-acetylcholine receptor antagonist with central selectivity, as a potential tool for generating a fast screening model of cognitive impairment, in outbred Wistar rats.

Methods We used several variants of the Morris water maze task: (1) reversal learning, to assess cognitive flexibility, with probe trials testing memory retention; (2) delayed matching to position (DMP), to evaluate working memory; and (3) "counter-balanced acquisition," to test for possible anomalies in acquisition learning. We also included a visible platform paradigm to reveal possible sensorimotor and motivational deficits.

Results A significant effect of biperiden on memory acquisition and retention was found in the counter-balanced acquisition and probe trials of the counter-balanced acquisition and reversal tasks. Strikingly, a less pronounced deficit was observed in the DMP. No effects were revealed in the reversal learning task.

Conclusions Based on our results, we do not recommend biperiden as a reliable tool for modeling cognitive impairment.

Keywords Anticholinergics · Muscarinic receptors · Learning · Memory · Morris water maze · Rat

Abbreviations

Ach	Acetylcholine
AChR	Acetylcholine receptors
AD	Alzheimer's disease
BBB	Blood-brain barrier
BIP	Biperiden
C	Control group
CA	Counter-balanced acquisition
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
DMP	Delayed matching to position
DMSO	Dimethyl-sulfoxide
GABA	Gamma-amino-butyric acid
GPCRs	G-Protein coupled receptors
i.p.	Intraperitoneally
ITI	Inter-trial interval
mAChR	Muscarinic acetylcholine receptors
MWM	Morris water maze
NSP	Non-spatial pretraining
s.c.	Subcutaneously
SCOP	Scopolamine
VP	Visible platform

Introduction

The rapid rise of incidence of neurodegenerative diseases in the aging population with no effective therapy available to date presses the need for the development of better animal models to be used in preclinical research. Here, we investigated the capacity of biperiden, an M1 selective antimuscarinic compound used in treatment of Parkinson's disease, to impair memory performance. This drug has been proposed as a

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potentially superior alternative to the non-selective scopolamine in generating fast screening animal models of neurodegeneration and dementia in rodents (Klinkenberg and Blokland 2011).

Klinkenberg and Blokland (2011) published a study which compared the effects of biperiden and scopolamine on various tasks using Skinner boxes, from operational conditioning to attention tests, and delayed non-matching to sample. Thus, short-term memory as well as food motivation and sensorimotor responsiveness could be evaluated, while enabling the authors to measure any potential attention deficit. Having tested three different doses of both drugs, the authors found short-term memory disruption following biperiden treatment (at doses of 1 and 3 mg/kg), whereas no significant changes in food motivation and attention were observed. Sensorimotor responding was affected only after the highest dose of 10 mg/kg. In contrast, scopolamine-treatment was followed by attention and sensorimotor deficits and lowered food motivation at both middle and high doses (0.3 and 1 mg/kg). Short-term memory was also affected; however, the authors argue the impairment may have been in a larger part of non-mnemonic nature. Thus, the authors conclude by recommending biperiden for future studies (Klinkenberg and Blokland 2011). In 2015, Blokland's group published another study assessing the effects of biperiden in human volunteers and validating biperiden as a translational modeling tool for research of cognition (Sambeth et al. 2015).

In contrast, the study of Szczodry et al. (2014) reached a virtually opposite verdict: a cone-field test revealed no significant differences between rats treated with biperiden (at both 3 and 10 mg/kg doses) versus controls, suggesting little or no influence on either working or reference memory. Furthermore, side effects were observed following the higher dose in the form of increased latency to start the task and lower number of food rewards collected, which might indicate possible xerostomia. Hence, in conclusion, the authors do not support the validity of this model for research of neurodegenerative diseases (Szczodry et al. 2014). Similarly, Talpos et al. (2014) found no effect of either scopolamine or biperiden on performance in a touch screen-based paired-associates learning which essentially evaluates spatial working memory.

Acetylcholine (ACh) counts among one of the major neurotransmitters and modulators in the nervous system; its receptors are abundantly expressed in a wide variety of tissues, from neuromuscular junctions and parasympathetic system to cortical regions involved in cognitive functions such as learning and memory (VanPatten and Al-Abed 2017). The cholinergic system has been shown to play an important role in processes such as circadian rhythmicity (Hut and Van der Zee 2011), addiction (Leslie et al. 2013), motivation, pain, and reward (VanPatten and Al-Abed 2017), as well as cognitive flexibility (Prado et al. 2017), perceptual memory (Robinson et al. 2011), spatial learning (Vales and Stuchlik 2005; Deiana et al. 2011), and many more. It comes as no surprise that any abnormalities in function of the cholinergic system and its components accompany a multitude of pathologies such as Parkinson's disease (Schliebs and Arendt 2011), Alzheimer's disease (Jiang et al. 2014), schizophrenia, bipolar disorder (Carruthers et al. 2015; Pittaras et al. 2016), and depression (Witkin et al. 2014). For these reasons, the cholinergic system has been extensively studied in the recent years; however, many mechanisms of its functioning remain mostly unclear. The main focus of this study, i.e., the target of the antimuscarinic biperiden, is the M1 receptor, which is considered to be the most abundant subtype (50–60% of all mAChR) of muscarinic receptors in the brain. It plays an essential role in many cognitive functions such as learning and memory and thus has become the target of research focusing on developing therapeutics for neurodegenerative diseases (Foster et al. 2014; Jiang et al. 2014; Carruthers et al. 2015). It is generally accepted that anticholinergics disrupt acquisition learning and long-term memory processing. As such, antimuscarinic compounds are often employed for inducing memory and cognitive impairments in laboratory animals in order to simulate pathological states observed in human diseases such as Alzheimer's disease and other dementias (Robinson et al. 2011). Detailed information regarding effect of antimuscarinic agents on various kinds of memory can be found in recent reviews (Deiana et al. 2011; Svoboda et al. 2017).

Biperiden hydrochloride (or lactate) is a proven M1-receptor selective antagonist. Approved for human usage and sold under the brand name of Akineton, it is prescribed for Parkinsonism (to improve motor abilities such as gait and tremor) and occasionally to suppress the side effects of neuroleptics (AHFS DI Essentials 2017). Due to its selectivity, it has been proposed as a potential tool for modeling cognitive impairment in rodents without sensorimotor side effects, specifically as a replacement for scopolamine, the so-called golden standard in research of cognitive impairment whose validity as a model has often been questioned on the grounds of its considerable side effects.

Scopolamine lacks selectivity for any of the subtypes of muscarinic receptors; apart from memory and cognition, it also affects sensorimotor, motivational, attentional, and other functions of the treated subjects, thus sometimes compromising the results of the behavioral tests (Klinkenberg and Blokland 2011). On the other hand, Robinson et al. (2004) reported an impaired performance in the Morris water maze (MWM) in both rats and mice following scopolamine administration at a dose that exhibited no effect on visual acuity (Robinson et al. 2004). Furthermore, Von Linstow Roloff et al. (2007) reported in their study that poor performance observed in scopolamine-treated rats in a delayed matching to place task in the MWM is in main part of the result of memory impairment, not due to compromised sensorimotor abilities (von Linstow Roloff et al. 2007). Scopolamine-

induced cognitive impairment was also shown to possess good validity as a translational model in research by our recent study (Laczó et al. 2017).

The up-to-date animal studies from rodents and even non-rodents using biperiden report conflicting results: while some authors observed clear disruption of learning and memory following biperiden treatment (Myers et al. 2002; Klinkenberg and Blokland 2011), others did not (Gieling et al. 2013; Talpos et al. 2014) or did only after an extremely high dose (Szczodry et al. 2014; Malikowska et al. 2017). Thus, we have decided to further investigate the matter by testing the properties of biperiden in several variants of the MWM. Specifically, we evaluated the effects of biperiden on acquisition, retention, and flexibility of spatial memory. We used a delayed matching to place version, reversal test, and counter-balanced acquisition in the MWM. We hypothesized that application of biperiden will impair delayed matching to place performance in Morris water maze with a longer delay (30 min), because this result would be in accordance with previous studies which tested effect of scopolamine on MWM task (von Linstow Roloff et al. 2007). We also predicted that biperiden application will induce a deficit in the memory retention and reversal, but will not cause deficit in the visible platform test.

Experimental procedures

Animals

The total of 90 male Wistar rats (2.5 months old, 270–450 g at the beginning of the experiments) obtained from the breeding colony of the Institute of Physiology of the Czech Academy of Sciences were used in this study. Two or three animals per cage were housed in transparent plastic cages (25 × 25 × 40 cm) without environment-enriching tools, and with water and feed available ad libitum. Cages were kept in an air-conditioned room with a constant temperature (21 °C), humidity (40%), and light-dark cycle 12/12 (at 6 a.m. switched on and 6 p.m. switched off). Separate groups of animals were used for different tasks employed in this study (i.e., reversal, delayed matching to place, and counter-balanced acquisition). The behavioral training took place between 8 a.m. and 5 p.m. (during the light part of the light/dark 12/12 cycle). The animals were handled in compliance with the Animal Protection Code of the Czech Republic and the corresponding directives of the European Community Council (2010/63/EC).

Drugs

The M1-selective muscarinic acetylcholine receptor antagonist biperiden hydrochloride (BIP; obtained from APExBIO, CZ) was first dissolved in dimethyl-sulfoxide (DMSO; 100 μl DMSO per 1 mg BIP) and then sterile saline (NaCl 0.9%) was added to reach the final concentration of 3 mg/ml. The solution was prepared a day before the drug treatment. Thirty minutes prior to testing, the rats were subcutaneously injected to the skin fold between their shoulders with either biperiden at a dose of 3 mg/kg, or a control solution consisting of DMSO in saline (300 μl DMSO per 1 ml saline).

Apparatus and behavioral procedures

The rats were trained in several versions of the MWM task (Czéh et al. 2001; Petrasek et al. 2014, 2016). The apparatus consisted of a pale blue pool (180 cm in diameter) filled to a depth of 28 cm with water (temperature approximately 22 °C) which was rendered opaque by addition of non-toxic black paint (Swingcolor, black). A transparent plastic escape platform (diameter = 10 cm) was placed in the pool (submerged 1 cm underwater), its position depending on the specific design of a given test. The surroundings of the pool provided an abundance of extra-maze cues usable for spatial learning and navigation. The rats' performances were recorded by an overhead camera connected to a tracking program (Tracker, Biosignal Group, USA). Facing the wall, a rat was released into the pool from four different locations (arbitrary south, east, north, and west) in a pseudorandom order (partial Latin square method). Rat was allowed freely to swim and its goal was to find the hidden platform using distal cues. If the animal failed to do so within 60 s from the start of each swim, it was gently guided to the platform. The rats were allowed approximately 15–30 s on the platform in order to memorize its position. In the reversal test and counter-balanced acquisition, rats were returned back to the cage for 10–15 min before the next trial started. In the DMP, there were four swims per day and the interval between the first and second swim was either 15 s or 30 min (randomized by a partial Latin square method). Moreover, intervals between the second and third and between the third and fourth swim was always 15 s just to maintain the win-stay strategy within a day (Steele and Morris 1999).

Reversal

The MWM reversal tests cognitive flexibility (Deiana et al. 2011; Prado et al. 2017), i.e., the ability to relearn a previously acquired task when the circumstances have slightly changed. The animals (controls: $n = 12$, biperiden-treated: $n = 10$) underwent 5 days of training with eight trials per day (Fig. 1). For the first 3 days (acquisition phase), the hidden platform was placed in the center of the north-east quadrant of the pool. For the remaining 2 days (reversal phase), it was repositioned in the southwest quadrant, and the rats received drug treatment. A probe trial was added at the end of the third, fourth, and fifth day to test memory retention; the platform was taken out of the pool and the rats were allowed to swim freely for a minute.

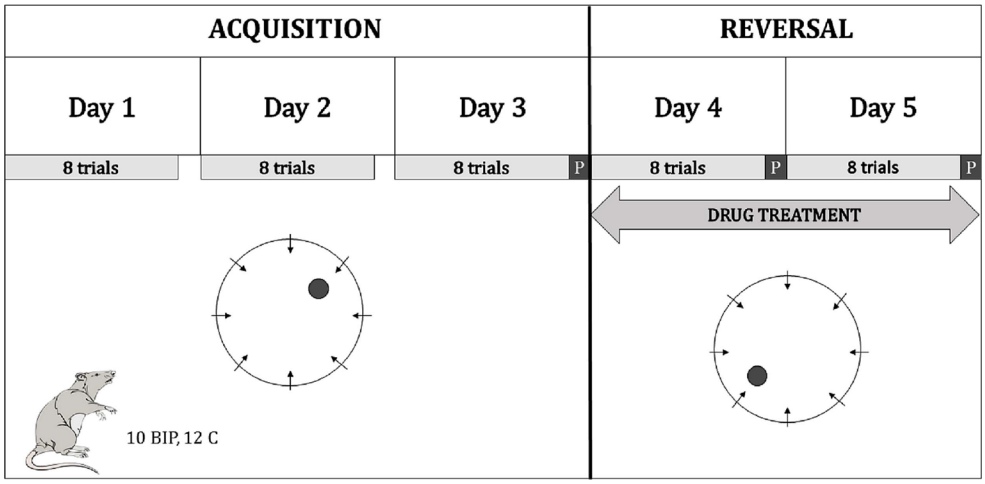

Fig. 1 Experimental design of the reversal task. The upper part shows a timeline (days 1–5) with the corresponding number of trials for each day (a dark box marked P stands for a probe trial). The double arrow denotes the days when the animals were subjected to drug treatment. The circles represent the pool, the position of the platform for the given set of days is marked by a filled circle, and the arrows signify the different starting positions. The rat in the bottom left corner stands for the total number of animals used in this task (i.e., 10 rats treated with biperiden, 12 rats treated with vehicle)

Delayed matching to position

This variant of the MWM tests working memory and memory trace persistence (von Linstow Roloff et al. 2007; Petrasek et al. 2014). Before the experiment itself, the animals (controls: $n = 21$, biperiden-treated: $n = 21$) underwent a 1-day non-spatial pretraining (NSP): any external cues were hidden by a black curtain and the rats were subjected to four swims, to become aware of the existence of the hidden platform and to get acquainted with the new settings. The DMP was then conducted over eight consecutive days with four trials per session, the position of the platform changing every day (Fig. 2). The rats were under drug treatment for the whole experiment (except for the non-spatial pretraining) and the inter-trial interval (ITI) between the first and second swim pseudorandomly changed between 15 s and 30 min each day for each animal. The DMP was followed by a visible platform test (VP), i.e., one session with four trials in which 1–2 cm part of the platform was visible above the water surface and was clearly marked with a ring and a hanging cue (a cross made out of two CDs on a string) for the rats to see.

The NSP part of the design ensures the rats are familiar with the settings and the existence of the hidden platform from the beginning of the DMP task itself. (However, given the actual performance in the DMP, the first 2 days of the DMP were left out of the statistical analysis, as 1 day of NSP was probably not sufficient for the animals to learn the task.) Due to the everyday changing of the platform position, the DMP presents a good tool for testing working memory. The subsequent 1-day four-trial visible platform task serves as a control of whether the results of the animals' performance might not have been influenced by, or due to a visual or motor impairment.

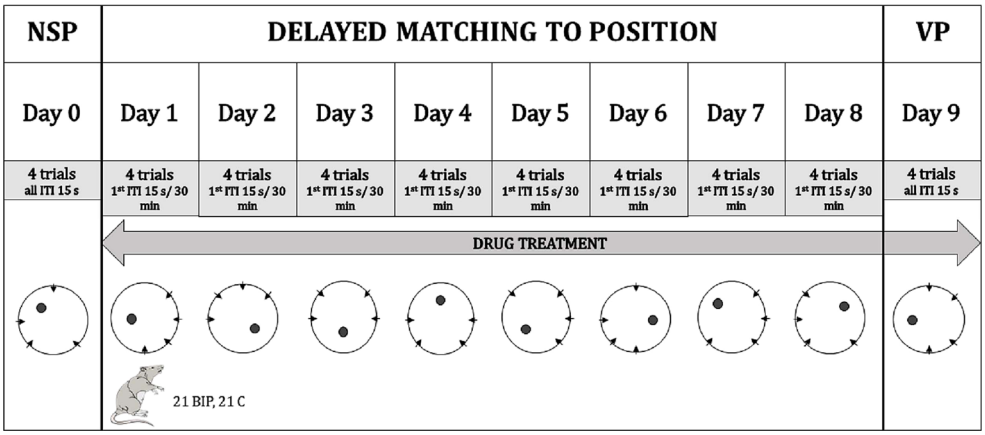

Fig. 2 Design of delayed matching to position task. For an explanation of the symbols used, see the commentary on Fig. 1

Counter-balanced acquisition

This design consisted of four consecutive testing days with eight trials per session and a probe trial at the end of the second and fourth day (Fig. 3). The position of the platform (NE) remained constant during the whole experiment. The rats were divided into two groups. The first group (B1; $n = 13$) received biperiden treatment for the first 2 days, whereas the other group (B2; $n = 13$) was treated with vehicle. For the remaining 2 days, the drug treatment was switched between the group, i.e., B1 were injected with saline + DMSO, and B2 with biperiden.

Measured parameters and statistical analysis

Data and analysis scripts can be found at https://osf.io/u48kv/. Number of animals in each experiment was based on previous studies using a similar experimental protocol. Given that neuroscience studies have been shown to often have limited statistical power (Button et al. 2013), it is possible that similar issues might apply even to our experiments. To partly overcome the issues, we tried to improve statistical power and precision of results by repeated measurement of dependent variables. We also report confidence intervals of estimated effect sizes, so that the uncertainty in the results caused by a relatively small number of animals can be easily seen.

Reversal

The analysis was conducted using mixed-effect regression (Gelman and Hill 2007). Mixed-effect regression can easily model repeated measures for a single subject as well as nested structure of data. In comparison to repeated measures analysis of variance, it has less strict assumptions and can fit data sets with missing data (Quené and Van Den Bergh 2004). Escape latency served as a dependent variable[1] and group (biperiden- or vehicle-treated), day, trial, and phase (acquisition or reversal) as well as their interactions served as predictors. Linear and quadratic contrasts were used for the effect of the trial. The effect of trial therefore estimated improvement within a day, its interaction with the effect of day estimated change in this improvement between days, and the main effect of day estimated improvement between days. The data analysis was conducted with an exclusion of the data from the first day which was regarded as required for learning the task. The remaining days were coded as 0.5 for the third day of acquisition and second day of reversal phase and − 0.5 for the second day of acquisition and first day of reversal. The subjects were nested within a run to take into account a possible dependence of data for subjects belonging to the same run. All analyses were conducted using R (R Core Team 2016).

Probe trials were analyzed with mixed-effect regression as well. The time spent in the target quadrant (i.e., the quadrant where the platform had been placed previously) served as a dependent variable and group (biperiden- or vehicle-treated) and day as well as their interaction served as predictors. Deviation coding was used for days. The subjects were nested within a run to take into account a possible dependence of data for subjects belonging to the same run.

Delayed matching to position

The analysis was conducted using mixed-effect regression. Escape latency served as a dependent variable and group (biperiden- or vehicle-treated), day, trial, and ITI served as predictors. Apart from the main effect of group, we also included its interaction with day, trial, and ITI in the model. Linear and quadratic contrasts were used for the effect of the day. For the effect of the trial, we used forward difference coding to test the changes between each two successive trials, and linear and quadratic contrasts to test the trend of changes between the trials. The subjects were nested within a run to take into account a possible dependence of data for subjects belonging to the same run. The data analysis was conducted with an exclusion of the data from the first 2 days which were regarded as required for learning the task.

Visible platform

The analysis of performance in this task was conducted similarly to the DMP, excluding the ITI and day predictors. We used polynomial contrasts for the trial effect.

Counter-balanced acquisition

We used mixed-effect regression for analysis of the latency to reach the platform. As predictors, we included the effect of group (biperiden administered the first 2 days vs. biperiden administered the last 2 days); the effect of biperiden, linear, and quadratic contrasts for effects of trial and day; the interactions of group effects, trial effects, and group; and the interaction between the effect of biperiden and trial effects. The model was selected by removing predictors from the full model based on the Akaike information criterion. We also nested the random effect for a subject under the effect of run.

For the probe trial results, the proportion of time spent in the target quadrant was analyzed using mixed-effect regression with the administration of biperiden and day as well as their interaction as predictors.

[1] While we had also data for the distance required to reach the platform, it correlated strongly with escape latency, $r(870) = .94$, 95% CI = [.94, .95], $p < .001$, so we show results only for escape latency for simplicity.

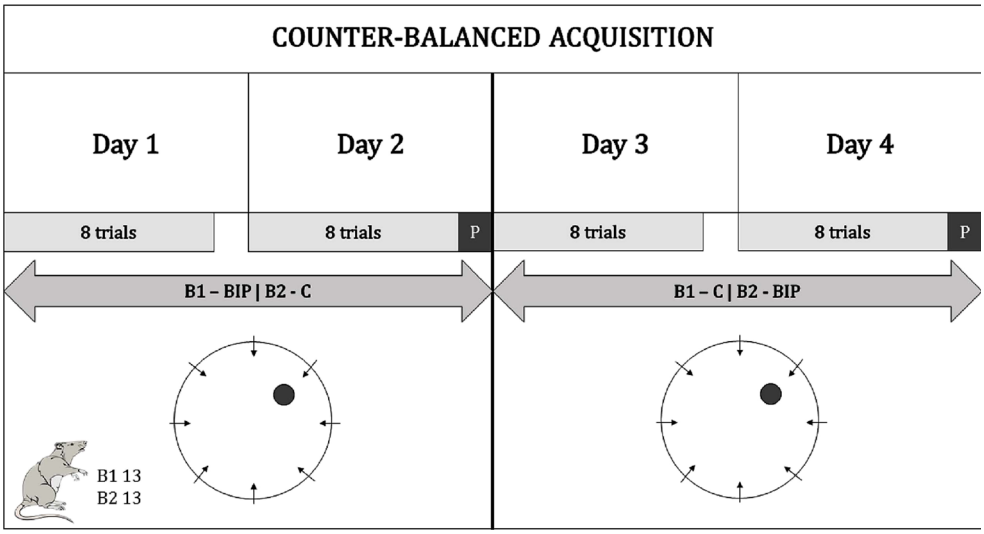

Fig. 3 Design of the counter-balanced acquisition test. For an explanation of the symbols used, see the commentary on Fig. 1. The double arrows denote what treatment each group of animals received for the given time span (bip biperiden, c control, i.e., vehicle)

Results

Reversal

Escape latencies were lower in the last days of a phase, $t(653.1) = -6.60$, $p < 0.001$, $b^2 = -8.21$, 95% CI = [− 10.65, − 5.78], but they did not differ between the two phases, $t(653.1) = -0.12$, $p = 0.91$, $b = -0.15$, 95% CI = [− 2.58, 2.29]. Escape latencies were shorter in later trials as suggested by the linear effect of a trial, $t(653.2) = -11.75$, $p < 0.001$, $b = -20.72$, 95% CI = [− 24.18, − 17.26], but the improvement was lower in later trials, $t(653.2) = 2.64$, $p = 0.008$, $b = 4.66$, 95% CI = [1.20, 8.12]. Most importantly, there was no effect of biperiden administration, $t(19.9) = -0.49$, $p = 0.63$, $b = -1.37$, 95% CI = [− 6.79, 4.05], as well as no interaction of biperiden administration with the effect of a day, $t(653.1) = 0.39$, $p = 0.70$, $b = 0.98$, 95% CI = [− 3.90, 5.85], phase, $t(653.1) = 0.26$, $p = 0.80$, $b = 0.64$, 95% CI = [− 4.24, 5.52], or linear effect of a trial, $t(653.2) = -0.99$, $p = 0.32$, $b = -3.49$, 95% CI = [− 10.40, 3.43]. The interaction between group and quadratic effect of trial was significant, $t(653.2) = -2.11$, $p = 0.04$, $b = -7.43$, 95% CI = [− 14.35, − 0.51], suggesting that biperiden-treated animals did not improve as much as animals in the control group with subsequent trials, but this effect was not specific just to the reversal phase where biperiden was administered. The linear effect of a trial was weaker in the last day of a phase, $t(653.1) = 3.45$, $p < 0.001$, $b = 12.15$, 95% CI = [5.24, 19.07] and this interaction was weaker in the reversal phase as suggested by the significant interaction of phase, day, and linear effect of a day, $t(653.1) = -2.13$, $p = 0.03$, $b = -15.03$, 95% CI = [− 28.86, − 1.21]. No other effect was significant (see Fig. 4a for the results).

[2] b refers to regression coefficient

Probe trials—reversal

Time spent in the target quadrant did not differ between the two groups, $t(54.7) = 0.57$, $p = 0.57$, $b = 1.99$, 95% CI = [− 4.82, 8.80], and it was not lower in the first day of reversal, $t(39.1) = -1.21$, $p = 0.24$, $b = -2.65$, 95% CI = [− 6.96, 1.66], or the second day of reversal, $t(39.5) = -0.29$, $p = 0.78$, $b = -0.64$, 95% CI = [− 5.00, 3.72], than in the last day of the acquisition phase. The difference between the last day of acquisition and the first day of reversal phase did not differ between the two groups, $t(39.1) = -1.44$, $p = 0.16$, $b = -6.35$, 95% CI = [− 14.97, 2.27], but it differed between the last day of acquisition and the second day of reversal, $t(39.5) = -2.47$, $p = 0.02$, $b = -10.97$, 95% CI = [− 19.70, − 2.25], showing that biperiden-treated animals stayed in the target quadrant for a shorter duration than the control animals in the second day of the reversal phase, $t(19) = -2.97$, $p = 0.008$, $d = -1.27$, 95% CI = [− 2.19, − 0.33], $M_{\text{biperiden}} = 22.05$ s, $M_{\text{control}} = 30.87$ s (see Fig. 5a for the results).

Delayed matching to position

There was no main effect of administration of biperiden on escape latency, $t(38.6) = 1.07$, $p = 0.29$, $b = 1.94$, 95% CI = [− 1.62, 5.49]. Escape latency decreased linearly with subsequent days, $t(950.2) = -3.24$, $p = 0.001$, $b = -4.47$, 95% CI = [− 7.16, − 1.77], but there was no quadratic effect of the day, $t(950.2) = 0.43$, $p = 0.67$, $b = 0.59$, 95% CI = [− 2.11, 3.30]. The improvement between days did not seem to level out within the 8 days of the experiment. The effect of the day also did not differ between the two groups either for the linear, $t(950.2) = 0.26$, $p = 0.80$, $b = 0.70$, 95% CI = [− 4.69, 6.10], or for the quadratic effect, $t(950.2) = -0.22$, $p = 0.83$, $b = -0.60$, 95% CI = [− 6.01, 4.81]. There was no effect of ITI on escape latency, $t(953.9) = 0.10$, $p = 0.92$, $b = 0.11$, 95% CI = [− 2.11,

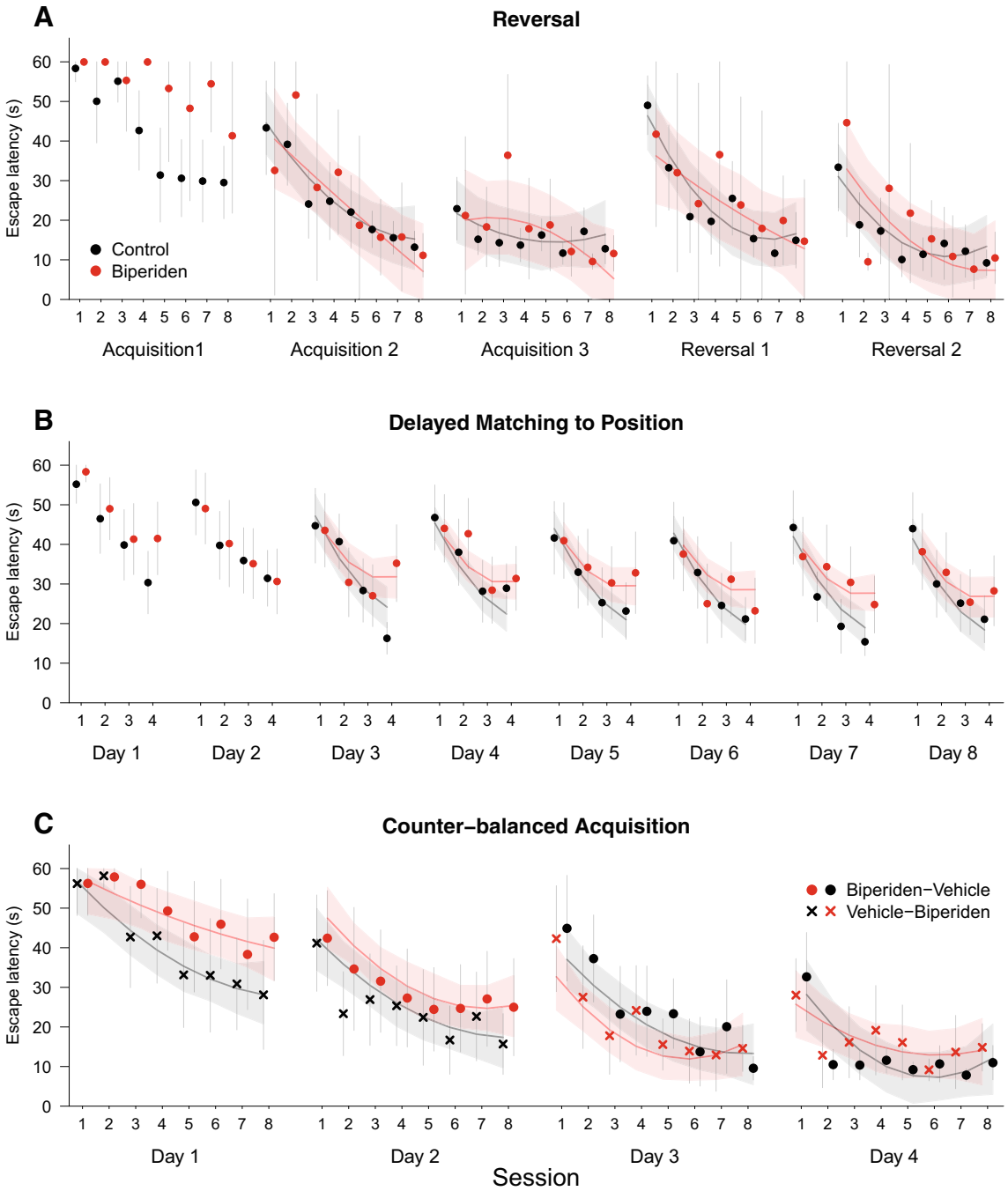

Fig. 4 Escape latencies in reversal, delayed matching to position, and counter-balanced acquisition tasks. Mean escape latencies for biperiden-treated animals (red) and control animals (black) are shown by points and the error bars represent 95% confidence intervals of the means. The solid lines represent predictions from the final models and the shaded regions represent 95% confidence intervals of the predicted means. Comparisons of predicted means from models to observed means suggest how well the models fit the data. Random effects were not included in the computation of confidence intervals and predictions from the models. **a** Mean escape latencies in reversal task. **b** Mean escape latencies in delayed matching to position task. Inter-trial intervals had virtually no effect and the model predictions are therefore shown only for one of the ITIs. The model with polynomial contrasts for trials was used for computation of the predictions. **c** Mean escape latencies in counter-balanced acquisition task

2.33], and no interaction of ITI with group was found as well, $t(953.8) = -0.16$, $p = 0.87$, $b = -0.36$, 95% CI = [−4.79, 4.07]. Escape latencies decreased between the first two trials, $t(950.3) = -5.38$, $p < 0.001$, $b = -8.57$, 95% CI = [−11.69, −5.45], and between the second and third trials, $t(950.2) = -4.04$, $p < 0.001$, $b = -6.42$, 95% CI = [−9.54, −3.30], but there was no further change between the last two trials, $t(950.2) = -1.14$, $p = 0.25$, $b = -1.82$, 95% CI = [−4.93, 1.29]. The two groups of rats did not differ significantly in the change of escape latencies between the first two trials, $t(950.3) = 0.99$, $p = 0.32$,

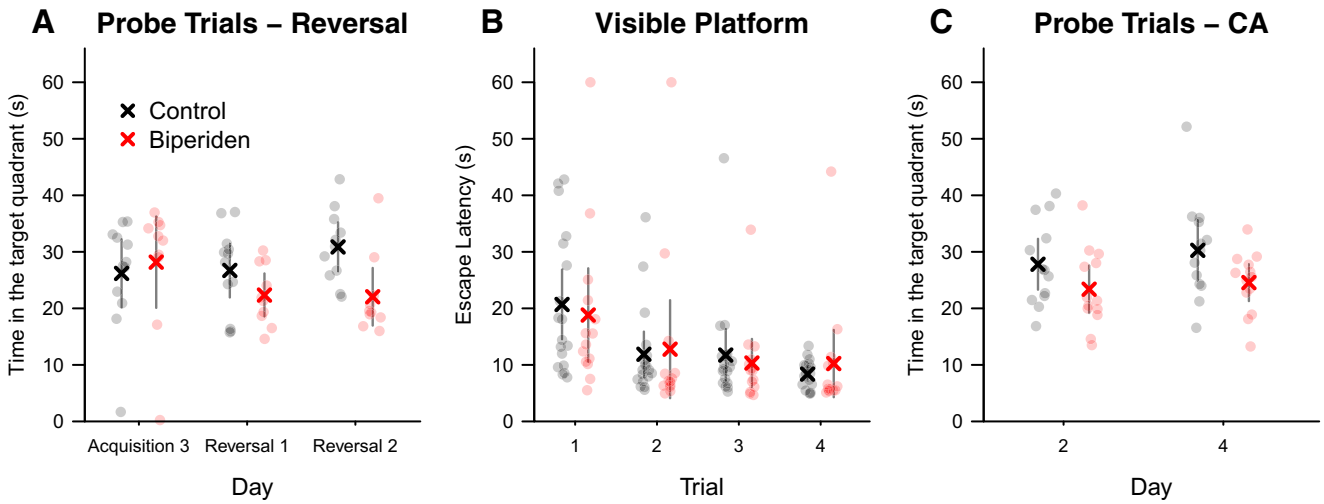

Fig. 5 Time in the target quadrant in probe trials and escape latencies in visible platform task. Mean escape latencies for biperiden-treated animals (red) and control animals (black) are shown by points and the error bars represent 95% confidence intervals of the means. The transparent points show all the observed data. **a** Mean escape latencies in probe trials following reversal task. **b** Mean escape latencies in visible platform task. **c** Mean escape latencies in probe trials following counter-balanced acquisition task

$b = 3.17$, 95% CI = [− 3.07, 9.41], between the second and third trial, $t(950.2) = 1.26$, $p = 0.21$, $b = 4.01$, 95% CI = [− 2.23, 10.24], and between the last two trials, $t(950.2) = 1.45$, $p = 0.15$, $b = 4.62$, 95% CI = [− 1.61, 10.85].

All the changes in escape latencies between trials were less marked for the biperiden-treated group. This can be seen when the analysis is done using polynomial contrasts for the trial effect instead of difference contrasts. Both linear, $t(952.2) = -3.25$, $p = 0.001$, $b = -4.46$, 95% CI = [− 7.16, − 1.77], and quadratic, $t(952.2) = 0.43$, $p = 0.67$, $b = 0.59$, 95% CI = [− 2.11, 3.30], contrasts for trials were significant. More importantly, the linear effect of the trial differed between the two groups, $t(952.2) = 3.92$, $p < 0.001$, $b = 8.81$, 95% CI = [4.41, 13.21], with the rats administered biperiden showing generally smaller decrease of escape latency within a session. The quadratic effect did not differ between the two groups, $t(952.2) = 0.32$, $p = 0.75$, $b = 0.73$, 95% CI = [− 3.68, 5.13]. The significant interaction of group with the linear effect of the trial suggests that biperiden-treated animals did not improve as fast as the control animals. When the analysis was done for each trial separately, biperiden-treated animals had somewhat lower escape latencies—even if not significantly—than in control animals in the first trial, $t(38.0) = -1.23$, $p = 0.22$, $b = -3.47$, 95% CI = [− 8.99, 2.04], and second trial, $t(38.4) = -0.23$, $p = 0.82$, $b = -0.64$, 95% CI = [− 6.20, 4.92], but they had higher escape latencies in the third trial, $t(40.0) = 1.31$, $p = 0.20$, $b = 3.61$, 95% CI = [− 1.81, 9.03], and significantly higher escape latencies in the fourth trial, $t(40.2) = 3.87$, $p < 0.001$, $b = 8.35$, 95% CI = [4.12, 12.58] (see Fig. 4b for the results).

Finally, we tested a specific prediction that biperiden would influence only long-term memory, which we tested by using only the change of escape latency between the first two trials for sessions with ITI of 30 min. The interaction between the effect of trial and group was not significant, $t(208.3) = 1.33$, $p = 0.19$, $b = 6.26$, 95% CI = [− 3.00, 15.51], suggesting that the rats treated with biperiden do not improve less in the trials with long ITIs.

Visible platform

The results showed a significant effect of both a linear, $t(92.0) = -6.23$, $p < 0.001$, $b = -7.30$, 95% CI = [− 9.60, − 5.00], and quadratic, $t(92.0) = 2.43$, $p = 0.02$, $b = 2.84$, 95% CI = [0.55, 5.14], contrasts for trial. Most importantly, the two groups did not differ in their performance, $t(30.0) = -0.04$, $p = 0.97$, $b = -0.13$, 95% CI = [− 6.21, 5.95], and unlike in the delayed matching to place task, they did not differ in their improvement within a session as well; $t(92.0) = 0.85$, $p = 0.40$, $b = 1.99$, 95% CI = [− 2.60, 6.59], for interaction with the linear contrast; and, $t(92.0) = 0.11$, $p = 0.91$, $b = 0.25$, 95% CI = [− 4.34, 4.85], for interaction with the quadratic contrast (see Fig. 5b for the results).

Counter-balanced acquisition

The analysis of the data showed that latency times decreased with subsequent days, $t(782.1) = -18.76$, $p < 0.001$, $b = -21.10$, 95% CI = [− 23.30, − 18.90], and trials, $t(782.1) = -11.70$, $p < 0.001$, $b = -18.61$, 95% CI = [− 21.73, − 15.49]. The quadratic contrast was significant for both days, $t(782.1) = 4.26$, $p < 0.001$, $b = 4.79$, 95% CI = [2.58, 6.99], and trials, $t(782.1) = 4.50$, $p < 0.001$, $b = 7.15$, 95% CI = [4.04, 10.27], suggesting that the improvement in escape latencies was stronger in initial days and trials than in later days and trials. The interaction of the linear effect of day and trial, $t(782.2) = 1.93$, $p = 0.05$, $b = 6.15$, 95%

CI = [− 0.10, 12.40], suggests that the improvement within a day decreased for later days.

Administration of biperiden did not influence escape latency times, $t(782.1) = -0.51$, $p = 0.61$, $b = -1.27$, 95% CI = [− 6.18, 3.65]. The order of administration of biperiden and saline did not have a significant effect on escape latency, $t(23.0) = 1.61$, $p = 0.12$, $b = 3.36$, 95% CI = [− 0.74, 7.47]. However, the interaction of group and the linear effect of a day was significant, $t(782.1) = -2.04$, $p = 0.04$, $b = -10.23$, 95% CI = [− 20.07, − 0.39], which shows that the group that was administered biperiden in the last 2 days improved less with subsequent days than the group that was administered biperiden the first 2 days. Given that the effect of day is confounded with the effect of biperiden administration, this suggests that biperiden administration had smaller effect in the group that was administered biperiden the last 2 days. This can be seen when the first 2 days and last 2 days are analyzed separately. Whereas for the first 2 days, biperiden-treated animals had significantly worse results than the control animals, $t(22.8) = 2.29$, $p = 0.03$, $b = 6.63$, 95% CI = [0.96, 12.31], there was no difference in the last 2 days, $t(24.1) = 0.03$, $p = 0.98$, $b = 0.07$, 95% CI = [− 5.00, 5.14] (see Fig. 4c for the results).

Probe trials—CA

The proportion of time spent in the target sector in probe trials was analyzed using mixed-effect regression with the administration of biperiden and day as predictors. The time spent in the target sector did not differ significantly between the 2 days with probe trials, $t(24.0) = 1.16$, $p = 0.26$, $b = 0.03$, 95% CI = [− 0.02, 0.08]. Administration of biperiden decreased the proportion of time spent in the target sector, $t(24.0) = -3.22$, $p = 0.004$, $b = -0.08$, 95% CI = [− 0.14, − 0.03]. The interaction between the day and administration of biperiden was not significant, $t(23.0) = -0.28$, $p = 0.78$, $b = -0.02$, 95% CI = [− 0.17, 0.13], that is, the effect of biperiden did not differ between the 2 days with probe trials (see Fig. 5c for the results).

Discussion

General remarks

In this study, we hoped to shed light on the matter of usability of biperiden as a cognitive impairer and help resolve the conflicting observations reported by other authors, using several design variants of the Morris water maze task to assess cognitive flexibility (reversal), working memory (DMP), memory acquisition (CA), and memory retention (probe trials included in reversal and CA experiments). We also conducted one session of visible platform paradigm to test for visual and/or sensorimotor impairment. Significant differences between the two experimental groups were found in the probe trials of both reversal and CA tasks, as well as in the first 2 days of the CA. Some differences were observed in the DMP as well; however, these were not clearly attributable to a working memory deficit. No significant differences were revealed in the reversal task. These results thus put our study somewhere in between the conflicting reports from other authors (Klinkenberg and Blokland 2011; Szczodry et al. 2014).

Cognitive flexibility and memory retention

In the *reversal* task, all rats successfully re-learned the new location of the hidden platform, suggesting no detrimental effect of biperiden on cognitive flexibility and adjusting to changed conditions once the principle of the task has been learnt. To the best of our knowledge, this is the first case of biperiden assessment in this task. Within the design of this paradigm, three probe trials were also conducted: (1) on the last day of acquisition phase, when no drugs had been administered, (2) and (3) at the end of the 2 days of reversal learning (under drug treatment). No differences in performance were found during the first (treatment-free) probe trial; however, in the very last probe trial (following drug injections), the biperiden-treated group was found to spend significantly less time in the quadrant where the platform had been previously positioned, hence suggesting memory retention impairment. These results were further confirmed in the probe trials conducted within the *counterbalanced* acquisition paradigm. The memory-retention impairment findings are in line with those of Gieling et al. (2013), who investigated the effects of biperiden in Gottingen minipigs in a hole-board task (Gieling et al. 2013), and the study of Kimura et al. (1999), focusing on alteration of performance in a step-down passive avoidance task (Kimura et al. 1999).

Working memory and persistence of memory trace

The delayed matching to position task was used to test for disruption of working memory. In agreement with the findings of Szczodry et al. (2014), Talpos et al. (2014), and partially of Gieling et al. (2013), we observed no markedly significant difference in performance between the biperiden-treated group and the control group, especially when comparing the rats' performance in the first two trials and regardless of the length of the first inter-trial interval. Although the biperiden group did exhibit a smaller decrease in escape latency times within a session, this can be attributed to the control animals' a priori displaying worse performance. A more detailed analysis revealed that the biperiden-treated animals performed as good as, or even better, than the control group in the first two trials. However, in the third and in the fourth trial, their escape latencies were higher than those of the control group (the difference being significant in the last trial) which might hint at a compromised memory processes.

Spatial task acquisition

In the *counter-balanced acquisition*, biperiden was found to significantly increase escape latency times when administered in the first 2 days, but not when administered in the last 2 days. In agreement with the work of (Kimura et al. 1999) and (Asth et al. 2012), these results suggest a disruptive effect of biperiden on memory acquisition. Although having investigated the binding properties of biperiden, Kimura et al. (1999) also reported a possible partial irreversibility of binding of this compound, which would explain longer-lasting effects observed in their study (Kimura et al. 1999). This might explain the lack of differences in performance between the two experimental groups during the last 2 days; possibly, the performance of the B1 group (who received biperiden injections for the first 2 days) was still compromised on the third and fourth day in spite of biperiden treatment cessation, whereas the B2 group (who were treated with biperiden for the last 2 days) worsened in their performance due to the biperiden injections.

Relation to other findings in rodent models

Taken together, our findings suggest only a minor effect of biperiden on spatial learning and memory, any disruption being perceptible only in memory retention and acquisition. However, in light of other studies reporting well-pronounced cognitive impairment following biperiden treatment, this compound cannot be simply ruled out as ineffective. There are many possible reasons for the contradictory results of our experiment and the work of Klinkenberg and Blokland (2011) and others. For example, Klinkenberg and Blokland (2011) reported using biperiden lactate which they dissolved in purified Milli-Q water and injected the animals intraperitoneally, whereas here, we used biperiden hydrochloride dissolved in DMSO (with saline added to reach the required concentration) and we administered the drug subcutaneously. Further, in aforementioned study, DMP task included appetitive food motivation in a Skinner box which is in contrast to aversive learning in our experiment. Szczodry et al. (2014) also argued their negative results may be due to the rat strain used; they chose Lister-Hooded rats for their experiment whereas Klinkenberg and Blokland (2011) used Wistar rats, who are known to be more sensitive to pharmacological interventions (Szczodry et al. 2014). Despite having used the Wistar strain as well, our findings are more in line with those of Szczodry et al. (2014). However, this does not entirely exclude the rat strain as one of the possible reasons for the differing results as long-term breeding in a single institution might over time generate differences even within a single strain. Other than that, the discrepancies in results may also be in part due to the particular behavioral tests employed, as each of them exhibits different sensitivity in revealing specific cognitive impairments.

Relation to findings in human subjects

Contrary to animal studies, human studies consistently show an impairment of memory after biperiden application. Memory impairment was observed in healthy volunteers, in elderly subjects, and also in patients with psychosis (Wezenberg et al. 2005; Sambeth et al. 2015; Borghans et al. 2017; Vingerhoets et al. 2017). In the light of present finding of relatively small detrimental effect of biperiden on memory in rat, question of translational validity of this model arises. In human data, most consistent effects were found in domains of episodic and verbal memory. DMP test is a test of one-trial-acquired memory, and in this way, it is similar to spatial episodic memory. Therefore, it is surprising that we observed no clear-cut and delay-dependent effect on DMP with biperiden. However, it is possible that DMP actually does not reflect an episodic-like memory task. With the present data, it is, therefore, difficult to provide a clear reconciliation of these differential effects in rats and humans. Nonetheless, it should be noted that a specific protocol in the MWM for rats and MWM-like real arena for humans has been developed recently and was successfully translationally validated with scopolamine and donepezil treatments (Laczó et al. 2017). To conclusively establish translational validity of biperiden, a similar human experiment would be beneficial.

Potential confounds and limitations

Regarding non-cognitive effects of biperiden, no differences were found in the visible platform paradigm, which suggests no visual impairment following biperiden injections. Average speed was also calculated for both experimental groups (data not shown), and again, no changes were revealed, pointing to little or no effect on motor skills. This is in contrast to the work of Asth et al. (2012) who reported the occurrence of hyperlocomotion in mice following biperiden treatment (Asth et al. 2012). The only observation of non-cognitive changes following biperiden treatment was when performing the experiment, the experimenter noticed a slightly increased anxiety-related behavior in the form of more frequent distress vocalization. This observation is similar to that of Szczodry et al. (2014), although they report increased fearfulness at a higher dose (10 mg/kg) (Szczodry et al. 2014).

Another aspect that might possibly play a role in the varying and sometimes conflicting results obtained by different laboratories is the previously mentioned complexity of the cholinergic system in the brain; mAChR are expressed both pre- and postsynaptically on various types of cells; hence, their activation might lead to diverse ends depending on timing and localization. In spite of being labeled as a predominantly postsynaptic receptor, in some cells, the M1-receptor may be found presynaptically as well, where it modulates activity of the given neuron (Kremin et al. 2006; Bell et al.

2013; Muller et al. 2013). For example, the M1 receptor (in cooperation with M2) has been shown to influence neurotransmission in the CA1 region of the hippocampus, where it suppresses glutamatergic signaling. It was suggested that this cholinergic activity probably forestalls older engrams from interfering during learning, and thus strengthen encoding and pattern discrimination (Kremin et al. 2006). Furthermore, presynaptic modulation by the M1-receptor has been hypothesized to be involved in processes of learning and memory, as it may stimulate glutamatergic transmission in hippocampal pyramidal cells (co-expressing NMDA receptors), consequently positively affecting long-term potentiation (LTP). A similar mechanism might also be employed in basolateral amygdala in fear conditioning (Muller et al. 2013).

An interesting hypothesis, which might be very relevant to this particular study, was proposed by Kremin et al. (2006). The authors argue that the M1 receptor may not be crucial to all tasks that are hippocampus-dependent; following a blockage of signaling via M1 receptor, the disrupted inhibition of interference of previously acquired memories might be perceivable only under certain conditions. For example, M1 knock-out mice have been shown to exhibit impaired performance in the radial-arm maze, possibly owing to the animals' inability to distinguish which arm they had already visited, and these circumstances change with each trial. In contrast, in the MWM, every trial contains the same, unchanging information (external cues, hidden platform) (Kremin et al. 2006). Hence, in our case, it may be possible that the MWM was not a sensitive enough task to reveal impairment caused by the biperiden M1 blockage.

The present study has several limitations. First is that we did not conduct a parallel experiment with application of scopolamine, an established behavioral impairer. This would allow for direct comparison of biperiden efficacy. The second limitation is that the experimenter was not blind to the treatment condition. The animal behavior was, however, tracked and analyzed digitally without an input of the experimenter. Last, we have employed only one dose of biperiden, i.e., 3 mg/kg; generalizability of the results to different doses therefore remains an open question. Nonetheless, the selection of the dose was based on previous studies, in which it was shown to work as a minimum dose with behavioral effects (Klinkenberg and Blokland 2011).

Concluding remarks

In this study, we investigated the effects of biperiden, an M1-selective muscarinic antagonist, which has been proposed as a potential tool for modeling cognitive impairment in rodents for the research of neurodegenerative diseases and preclinical testing in drug development. To this end, we used several variants of the Morris water maze, which assess different components of learning and memory: (1) cognitive flexibility, tested in reversal learning, and (2) working memory, vital for the DMP task, were unimpaired in the biperiden-treated animals. An increase in escape latency following biperiden injections was observed during the first 2 days in (3) acquisition learning (in the CA task). A significant impairment of (4) reference memory was revealed in the probe trials of the reversal and CA tasks. Also, the biperiden-treated rats displayed smaller improvement within the four trials each day in the DMP which may have been either due to the worse performance of the control group in the first two trials or possibly due to memory impairment. Based on our results, biperiden seems to exert some influence on cognitive processes involved in spatial navigation; however, these were not markedly clear with the given number of subjects. It is possible, given the complexity of the muscarinic cholinergic system in the brain, that the MWM is not a task well-suited to assessment of the consequences of this particular M1 blockade. The effects might be more perceptible and clear-cut if a larger number of experimental subjects was used. However, taking into account the ethics of working with laboratory animals, such a course of action would be at the very least questionable. Notwithstanding, the varying results reported by different laboratories make it rather unreliable as a research tool. As a number of other means of modeling dementia and cognitive deficits in rodents may be employed, we would thus not recommend biperiden as a useful cognitive impairer for preclinical research of dementia and cognitive deficits.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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