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ORIGINAL INVESTIGATION

A comparison of scopolamine and biperiden as a rodent model for cholinergic cognitive impairment

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Abstract
Rationale The nonselective muscarinic antagonist scopolamine hydrobromide (SCOP) is employed as the gold standard for inducing memory impairments in healthy humans and animals. However, its use remains controversial due to the wide spectrum of behavioral effects of this drug.
Objective The present study investigated whether biperiden (BIP), a muscarinic m1 receptor antagonist, is to be preferred over SCOP as a pharmacological model for cholinergic memory deficits in rats. This was done by comparing the effects of SCOP and BIP using a battery of operant tasks: fixed ratio (FR5) and progressive ratio (PR10) schedules of reinforcement, an attention paradigm and delayed nonmatching to position task.
Results SCOP induced diffuse behavioral disruption, which included sensorimotor responding (FR5, 0.3 and 1 mg/kg), food motivation (PR10, 1 mg/kg), attention (0.3 mg/kg, independent of stimulus duration), and short-term memory (delayed nonmatching to position (DNMTP), 0.1 and 0.3 mg/kg, delay-dependent but also impairment at the zero second delay). BIP induced relatively more selective deficits, as it slowed sensorimotor responding (FR5, 10 mg/kg) and disrupted short-term memory (DNMTP, 3 mg/kg, delay-dependent but no impairment at the zero second delay). BIP had no effect on food motivation (PR10) or attention.
Conclusion Muscarinic m1 antagonists should be considered an interesting alternative for SCOP as a pharmacological model for cholinergic mnemonic deficits in animals.

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Introduction

The muscarinic antagonist scopolamine hydrobromide (SCOP) is used as the gold standard for inducing deficits in human and animal models of memory dysfunction. Justification for this purpose has been provided by the cholinergic hypothesis of geriatric memory dysfunction proposed in the early 1980s by Bartus et al. (1982). The SCOP model is still used extensively for preclinical testing of new substances designed to treat cognitive impairment (e.g., Barak and Weiner 2009; Buccafusco et al. 2008; Cunha et al. 2008; Loiseau et al. 2008; Vaisman and Pelled 2009). However, its use in cognition research is surrounded by controversy (Hodges et al. 2009; Klinkenberg and Blokland 2010). SCOP is nonselective in terms of binding affinity and, depending on its dose, has the capability to block cholinergic neurotransmission at all muscarinic receptor subtypes m1–m5 (Bolden et al. 1992; Bymaster et al. 2003). As muscarinic receptors are found throughout the brain and body (Caulfield 1993), SCOP is able to induce widespread effects.

Systemic injections of SCOP are capable of disrupting several autonomic nervous system functions. At doses of 0.01 mg/kg and higher, SCOP can reduce salivation ("dry mouth" side-effect, Dai et al. 1991; Hodges et al. 2009; Shiraishi and Takayanagi 1993), which may lower responding in tasks which employ solid food rewards in order to motivate the animals. Although this problem can be dealt with by using liquid reinforcers (Hodges et al. 2009), the

majority of studies still favor dry food pellets (Klinkenberg and Blokland 2010). Furthermore, most behavioral tasks also have a strong noncognitive component which can be influenced by SCOP treatment (e.g., increases in locomotor activity, response latency, and omissions at doses lower than 0.03 mg/kg, Bushnell et al. 1997; Klinkenberg and Blokland 2010; Mirza and Stolerman 2000; Phillips et al. 2000; Sipos et al. 1999). Performance on behavioral tasks assessing sensory/stimulus discrimination and/or attentional processes appears to be most susceptible to SCOP treatment (Hodges et al. 2009). Only if doses higher than 0.1 mg/kg are administered systemically, robust performance deficits on a variety of learning and memory tasks are reported (Klinkenberg and Blokland 2010). Therefore, it has been argued that acetylcholine is predominantly involved in mediating discriminatory and attentional processes (Blokland 1995; Everitt and Robbins 1997; Fibiger 1991; Sarter and Bruno 1997) rather than learning and memory functions (Bartus et al. 1982). In sum, the validity of SCOP as a tool for inducing cognitive dysfunction is questionable.

Of note, systemic administration makes it difficult to dissociate central and peripheral effects. One way to address this issue is to include an experimental group that is given methyl-scopolamine, a quaternary form of SCOP that has the same receptor binding characteristics but supposedly does not cross the blood–brain barrier when given at an equivalent dose (Evans 1975; Harvey et al. 1983; Pradhan and Roth 1968). However, several animal studies have shown that methyl-scopolamine can influence measures of cognitive performance (e.g., Andrews et al. 1994; Herremans et al. 1995; Moore et al. 1992; Pakarinen and Moerschbaecher 1993; van Haaren and van Hest 1989). In addition, methyl-scopolamine cannot control for the widespread blockade of central muscarinic receptors after systemic administration of SCOP (Frey et al. 1985).

Several of the muscarinic receptor subtypes m1–m5 might underlie the cognitive effects of SCOP. Muscarinic m2 presynaptic autoreceptors have an inhibitory effect on acetylcholine efflux (Bymaster et al. 2003). Hence, m2 antagonists might act as cognitive enhancers by elevating central cholinergic tone, but behavioral data are mixed (Carey et al. 2001; Daniel and Dohanich 2001; Messer and Miller 1988; Quirion et al. 1995). The role of m3 receptors in cognition is as of yet quite obscure (Bymaster et al. 2003), although one study showed a potential role of m3 receptors in cognition (Poulin et al. 2010). Information on the involvement of m4 presynaptic autoreceptors and m5 postsynaptic in cognitive function is also relatively limited (Wess 2004); there are some indications that the m5 receptor is implicated in central cerebral blood flow and memory processes (Araya et al. 2006).

Evidence for a role in mnemonic processes in both rodents and humans is strongest for the postsynaptic muscarinic m1 receptor (e.g., Conn et al. 2009; Fornari et al. 2000; Kimura et al. 1999; Kramer-Soares et al. 2006; Roldán et al. 1997; Wezenberg et al. 2005). This receptor is predominantly located in brain regions thought to be important for learning and memory such as cortex and hippocampus; presence of the m1 receptor in the periphery is relatively limited (Caulfield 1993; Volpicelli and Levey 2004). Hence, m1 antagonists are considered an interesting option with regards to finding novel pharmacological alternatives to induce cognitive impairment which are not so much hampered by issues of nonselectivity or peripheral side-effects (Conn et al. 2009).

This is the first study to compare the effects of SCOP versus the relatively more selective muscarinic m1 antagonist biperiden (BIP) (Bolden et al. 1992) on various facets of behavior. Specifically, we wanted to dissociate behavioral effects of these two drugs on a battery of four operant tasks: fixed ratio (FR5) and progressive ratio (PR10) schedules of reinforcement (assessing sensorimotor responding and food motivation, respectively) versus performance in an attention task and a delayed nonmatching to position task (assessing short-term memory). On basis of the direct comparison between both drugs we wanted to determine whether BIP would be preferable over SCOP as a cholinergic memory deficit model.

Methods

Subjects

All experimental procedures were approved by the local ethical committee for animal experiments at Maastricht University and met governmental guidelines. Twenty male 3-month-old Wistar rats (Harlan, NL) served as subjects in this study. To ensure consistency, the same animals were used in all behavioral tasks. They were housed in pairs in standard type III Makrolon™ cages on sawdust bedding in an air-conditioned room (21°C, 45–55% humidity) under a reversed light/dark cycle (lights on from 7 P.M. to 7 A.M.). Rats were housed in the room in which they were tested. All testing was performed between 12 and 6 P.M. Rats had free access to water, but were subjected to a food deprivation regime from Monday through Friday, in order to reduce their weight to about 90% of their free feeding weight. Food was given ad libitum from Friday afternoon to Sunday afternoon. Food was taken away at Sunday afternoon which caused a sufficient appetite at the morning session on Monday.

Apparatus

Rats were trained and tested in 10 identical Skinner boxes (40×30×33 cm). The ceiling of these conditioning cham-

bers contained a light that illuminated the conditioning chamber during experiments. The left and right sidewalls served as control panels. A recess (5×5 cm), built into the left side panel 2.5 cm above the grid floor, contained a food tray with a hinged panel into which a pellet dispenser delivered 45-mg food pellets (Bioserve TestDiet AIN-76A rodent tablets, Frenchtown, NJ, USA). Two retractable stainless steel levers (4 cm wide) projected 2 cm into the conditioning chamber and were located 6 cm from both sides of the recess, 12 cm above the grid floor. The conditioning chambers were enclosed in sound-attenuating housing. Background noise was produced by a radio and an exhaust fan. A personal computer controlled the experimental equipment and collected the data.

Fixed ratio (five) task

Rats first underwent five magazine training sessions and were then subjected five times to continuous reinforcement (CRF). Next the rats were trained on a fixed ratio schedule of reinforcement, in which they had to press a lever for five times (FR5) in order to obtain a 45-mg food reward. Reinforcement was continuous; i.e., each set of five lever presses was rewarded. A session was terminated after 60 trials or 30 min, whichever came first. Rats were trained once a day, Monday to Friday, and were given eight FR5 sessions before drug testing started. The measure used to evaluate performance on the FR5 schedule was inter-response time (i.e., time between consecutive lever presses which was averaged for each animal).

Progressive ratio (ten) task

After finishing drug testing in the FR5 task, rats immediately started training on a progressive ratio (PR10) schedule of reinforcement (Hodos 1961). PR tasks are generally used to assess the reinforcing efficacy of a particular type of reward. The rats had to progressively increase the response requirement (steps of ten lever presses) to obtain a food reward. For the first food pellet they were required to press ten times, for the next reinforcement they had to press the lever twenty times, and so on. A session was terminated if a rat did not press the lever for 3 min. Rats were trained once a day, Monday to Friday, and were given eight PR10 sessions before drug testing started. The measure used to evaluate performance in the PR10 task was breakpoint (i.e., number of lever presses made during a session).

Attention task

After the PR10 task, the rats were subjected to one CRF session before they started training in an attention task. During this task, a light stimulus was presented either on the left side or on the right side of the food reward tray. The duration of the light stimulus varied randomly between 3, 1 and 0.3 s. One second after the light stimulus was extinguished, the two levers were inserted simultaneously. When the rat hit the lever on the side of the prior light stimulus (*correct response*), the rat was rewarded with a food pellet followed by an inter-trial interval of 5–10 s. When the rat hit the lever on the opposite side of the previous light stimulus (*incorrect response*), the rat was not rewarded and a time-out period of 5 s was followed by an inter-trial interval (ITI). When the rat did not hit a lever within 3 s (*omission*), the rat was not rewarded and both levers were retracted followed by a time-out period of 5 s and an ITI. A session was terminated after 80 trials or 40 min, whichever came first. A more detailed description of this task is provided by Hoff et al. (2007). Rats were trained once a day, Monday to Friday. The derived behavioral measures were percentage correct, percentage omissions, response time (averaged over all stimulus durations) and two signal detection theory derived measures:

1. *The sensitivity index (SI):* a signal detection measure for discriminability which was calculated as follows: $SI = (h-f)/(2(h+f)-(h+f)2)$, where h=(correct left)/(correct left+incorrect right) and f=(incorrect left)/(incorrect left+correct right). A value of zero reflects no discrimination whereas a value of 1 reflects perfect discrimination.
2. *Index Y:* a signal detection derived variable for evaluating a response bias. This parameter is calculated as follows: (percentage correct left − percentage correct right)/(percentage correct left + percentage correct right).

A more detailed description of SI, index *Y*, and other signal detection measures can be found elsewhere (Steckler 2001).

Delayed nonmatching to position

After the rats had finished drug testing on the attention task, they immediately started training in a nonmatching to position task, to which subsequently delays were added. This paradigm consisted of two stages: a sample and a choice phase. In the sample phase, one of the two levers was inserted into the operant chamber. After the rat had pressed the sample lever, it was retracted and the rat was required to poke its nose against the hinged panel which gave access to the pellet magazine (positioned equidistantly between the two levers). This was done in order to prevent the rats from using a behavioral strategy (i.e., mediating behavior, Herremans and Hijzen 1997) to perform the delayed nonmatching to position (DNMTP) task (e.g., after pressing a lever they can move to the other side and wait

for the lever to come out). More than one panel press or keeping the nose in the food tray was without consequence.

After the rat had pushed the panel at least once and had pulled its nose out of the food hopper, both levers were inserted (the choice phase) and the rat was required to press the lever opposite to the one in the sample phase. It was physically not possible for an animal to keep its nose in the pellet magazine and press the levers, as the food hopper and response levers were placed too far apart. A nonmatching lever-press was continuously reinforced with a food reward. There was a 5-s time-out period (and no food reward) when the lever pressed in the "choice" phase was the same one as in the "sample" phase (i.e., when the response was incorrect). The ITI was always 8 s (also in subsequent testing in the DNMPT task). A session was terminated after 80 trials or 60 min, whichever came first. No limited hold period was used for the sample or choice phase, which means that no omission errors were recorded. A more elaborate description of the nonmatching to position (NMTP) training can be found elsewhere (Blokland et al. 2004; Prickaerts et al. 1999).

Rats were trained once a day, Monday to Friday and received five NMTP training sessions and one forced choice NMTP session (in which the task was continued only after a correct response was given) before delay intervals were being introduced in between the sample and choice phase. The duration of the delays was gradually increased over successive training sessions over a period of about 2.5 weeks. In order to speed up DNMTP training rats now received two daily sessions. The delay interval was randomly chosen from the following five alternatives: 0, 2, 4, 8, or 16 s. The animals were able to keep their nose in the food hopper during the delays or press the food panel repeatedly without any consequence, but could not press the levers as these were retracted during the delay. In previous studies, we have not observed the animals developing a mediating strategy while performing the DNMTP task (e.g., Blokland et al. 2004), as they were required to press the panel of the food well before the choice phase was presented. The measures used to evaluate performance in the DNMTP were percentage correct, response time (averaged over all delays), SI and index *Y* (see "Attention task" for more information on these last two parameters).

Drug treatment

Dose range and pretreatment time were chosen based on previous SCOP and BIP data (e.g., Hodges et al. 2009; Jones and Shannon 2000). Dose conditions were determined according to their position on a logarithmic scale. For example, BIP doses were 1, 3, and 10 mg/kg. When converted to logarithms, these values are approximately equally spaced: 0.0, 0.5, and 1.0, respectively. Doses were titrated on basis of behavioral effects found in our essay. Scopolamine hydrobromide trihydrate 99% (hereafter abbreviated as SCOP, obtained from Acros Organics) was dissolved in isotonic saline in doses 0, 0.1, 0.3, and 1 mg/kg (milligrams salt per kilogram of body weight), whereas biperiden lactate (hereafter abbreviated as BIP, Akineton® obtained from Laboratorio Farmaceutico S.I.T.) was dissolved in Milli-Q purified water in doses 0, 1, 3, and 10 mg/kg (milligrams salt per kilogram of body weight). We used quite high doses of SCOP and BIP (1 and 10 mg/kg, respectively) as an upper limit at which—certainly in case of SCOP—serious behavioral side-effects were expected. All drug solutions were prepared freshly each day prior to testing. SCOP and BIP were both injected in a volume of 2 ml/kg (IP) with a pretreatment time of 30 min. Each drug dose was tested once per rat per test. On each testing day, only one SCOP and one BIP dose was given, with half of the rats receiving SCOP and the other half receiving BIP. The order of doses was semi-randomized over testing days.

Repeated testing

Repeated testing of drugs in the same group of animals offers several advantages over between-group studies (e.g., better statistical power). However, this particular type of design can be associated with tolerance, drug sensitivity and carry-over effects. To ensure sufficient wash-out of the drug, testing days were always separated by at least one drug-free day on which the animals received FR5, PR10, attention task or DNMTP training. Frequency of administration and dose level were kept as low as possible (i.e., no higher doses were tested than those yielding a significant behavioral effect). This procedure minimized the number of injections each rat received. In order to further minimize group differences due to receptor changes, the drug that was given (SCOP or BIP) alternated between groups for the different behavioral tasks; i.e., ten rats received only SCOP doses and the other ten only received BIP doses during testing of one paradigm. When testing of the next behavioral test started this order was reversed: rats which had previously received SCOP, now received BIP and vice versa.

Statistical analysis

Data were analyzed by parametric analysis of variance (mixed model analysis of variance (ANOVA); SPSS 15.0) with dose as within-subject variable and drug as between-subject variable. In case an interaction with drug and/or a main effect of drug was found, a repeated measures ANOVA was performed for each drug separately, with dose as within-subject variable (and possibly stimulus duration

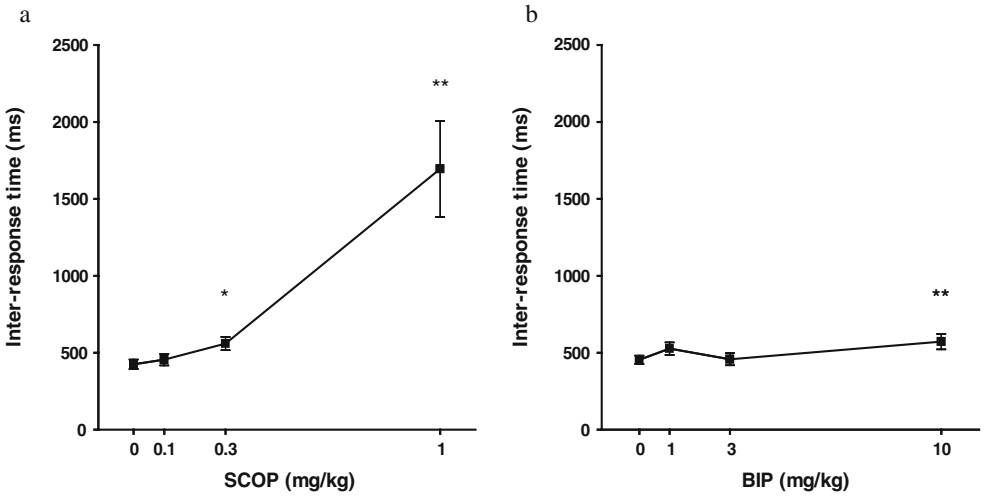

Fig. 1 The effects of SCOP (0.1, 0.3, and 1 mg/kg, IP) and BIP (1, 3, and 10 mg/kg, IP) on a FR5 schedule of reinforcement. **a**, **b** Inter-response time. SCOP slowed sensorimotor responding at a dose of 0.3 and 1 mg/kg. BIP slowed sensorimotor responding at a dose of 10 mg/kg. Data represent mean (+SEM). *Asterisks* indicate differences from vehicle condition (*P<0.05; **P<0.01)

or delay). Hence, drug effects of SCOP and BIP were compared with their own vehicle condition: i.e., SCOP with saline and BIP with Milli-Q. For the analysis of the attention task and the DNMTP, stimulus duration and delay were added as additional within-subject variables, respectively. In case a significant dose×stimulus duration or dose×delay interaction was reported, several repeated measures ANOVAs were run separately for stimulus duration or delay, respectively. One exception was the measure response time; here, data were averaged for each animal and collapsed across stimulus duration or delay. Differences from vehicle conditions were always examined with a least significant difference post hoc test. Due to some mechanical issues, occasionally data of nine rats were used for analysis.

Results

Fixed ratio (five) task

Three rats failed to complete 60 trials within 30 min after a dose of 1 mg/kg SCOP. Figure 1 shows the effects of SCOP and BIP on inter-response time in a FR5 schedule of reinforcement. In the mixed model ANOVA, the within-subject effect of dose on inter-response time varied per level of drug (dose×drug interaction effect; $F(3, 48)=10.77$; $P<0.001$). Therefore two separate repeated measures ANOVAs for the different levels of drug were performed. For the group treated with SCOP, inter-response time in the FR5 task was increased (main effect of dose; $F(3, 24)=12.82$; $P<0.001$; see Fig. 1a). Post hoc analysis showed that the 0.3 ($P<0.05$) and 1 mg/kg ($P<0.01$) doses slowed responding. In the group treated with BIP, there was an increase in FR5 inter-response time (main effect of dose; $F(3, 24)=7.80$; $P<0.01$; see Fig. 1b). Post hoc analysis indicated only an effect of the high 10 mg/kg dose ($P<0.01$)

Progressive ratio (ten) task

Figure 2 shows the effects of SCOP and BIP on breakpoint and inter-response time on a PR10 schedule of reinforcement. In the mixed model ANOVA, the within-subject effect of dose on breakpoint did not vary per level of drug (no dose×drug interaction effect; $F(2, 36)=0.77$, n.s.). Furthermore, breakpoint was differentially affected by dose (main effect of dose; $F(2, 36)=5.57$; $P<0.01$). The between-subject analysis of drug showed that SCOP and BIP differentially affected breakpoint (main effect of drug; $F(1, 18)=5.93$; $P<0.05$). Therefore, two separate repeated measures ANOVAs for the different levels of drug were performed. For the group treated with SCOP, breakpoint in the PR10 task was reduced (main effect of dose; $F(2, 18)=3.91$; $P<0.05$; see Fig. 2a). Post hoc analysis showed an effect of the 1 mg/kg dose ($P<0.05$). For the group treated with BIP, no change in breakpoint (no main effect of dose; $F(2, 18)=2.10$, n.s.; see Fig. 2b) was found.

In the mixed model ANOVA, the within-subject effect of dose on inter-response time did not vary per level of drug (no dose×drug interaction effect; $F(2, 36)=0.38$, n.s.; see Figs. 2c and 2d). The within-subject analysis of dose was not significant (no main effect of dose; $F(2, 36)=3.04$, n.s.), which means that the different dose conditions also did not change inter-response time. The between-subject analysis of drug showed that SCOP and BIP did not differentially affect inter-response time (no main effect of drug; $F(1, 18)=0.32$, n.s.).

Attention task

Figure 3a, b shows the effects of SCOP and BIP on percentage correct in the attention task. In the mixed model ANOVA, the within-subject effect of dose on percentage correct did not vary per level of drug and stimulus duration (no dose×drug×stimulus duration interaction effect;

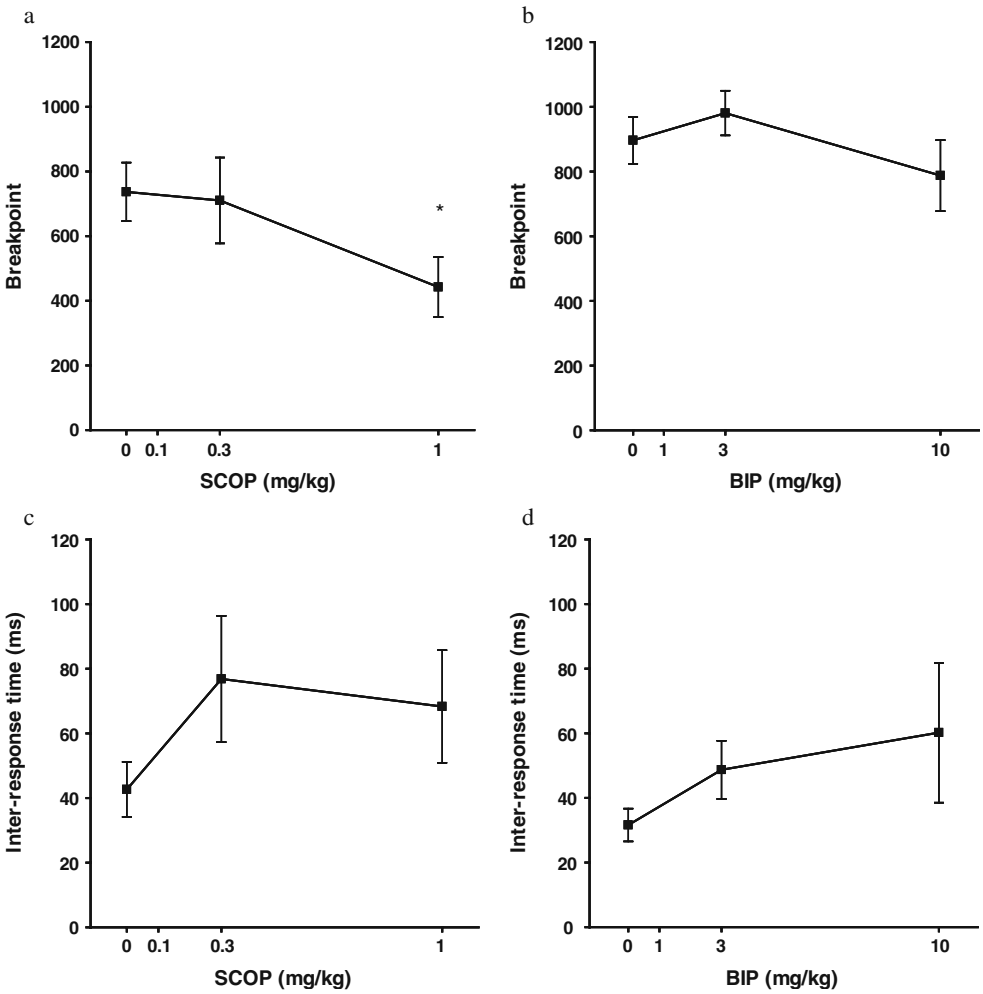

Fig. 2 The effects of SCOP (0.3 and 1 mg/kg, IP) and BIP (3 and 10 mg/kg, IP) on a PR10 schedule of reinforcement. **a**, **b** Breakpoint. SCOP decreased food motivation at a dose of 1 mg/kg. BIP did not have an effect on food motivation. **c**, **d** Inter-response time. SCOP slowed sensorimotor responding at a dose of 1 mg/kg. BIP did not have an effect on sensorimotor responding. Data represent mean (+SEM). *Asterisks* indicate differences from vehicle condition (*$P<0.05$)

$F(4, 68)=0.75$, n.s.). However, the within-subject effect of dose on percentage correct did vary per level of drug (dose×drug interaction effect; $F(2, 34)=7.96$; $P<0.01$). The within-subject effect of stimulus duration on percentage correct was also different per level of drug (stimulus duration×drug interaction effect; $F(2, 34)=3.82$; $P<0.05$). Therefore separate repeated measures ANOVAs for the two levels of drug were performed. In the group treated with SCOP, the within-subject effect of stimulus duration on percentage correct was not different per level of dose (no stimulus duration×dose interaction effect; $F(4, 36)=0.83$, n.s.; see Fig. 3a). There was a reduction in percentage correct responses with shorter stimulus durations (main effect of stimulus duration; $F(2, 18)=40.06$; $P<0.001$). SCOP decreased percentage correct responses in the attention task (main effect of dose; $F(2, 18)=20.55$; $P<0.001$). Post hoc analysis showed that at a dose of 0.3 mg/kg SCOP lowered percentage correct score as compared with the vehicle condition ($P<0.01$). In the group treated with BIP, the within-subject effect of stimulus duration on percentage correct was not different per level of dose (no stimulus duration×dose interaction effect; $F(4, 32)=0.36$, n.s.; see Fig. 3b). Moreover, shorter stimulus durations reduced percentage correct (main effect of stimulus duration; $F(2, 16)=149.29$; $P<0.001$). BIP treatment did not affect the measure percentage correct (no main effect of dose; $F(2, 16)=2.69$, n.s.).

Figure 3c, d shows the effects of SCOP and BIP on percentage omissions in the attention task. In the mixed model ANOVA, the within-subject effect of dose on percentage omissions did not vary per level of drug and stimulus duration (no dose×drug×stimulus duration interaction effect; $F(4, 68)=0.98$, n.s.). The within-subject effect of stimulus duration on percentage omissions was also not different per level of drug (no stimulus duration×drug interaction effect; $F(2, 34)=1.16$, n.s.). In contrast, the within-subject effect of dose on percentage omissions did vary per level of drug (dose×drug interaction effect; $F(2, 34)=6.98$; $P<0.01$). Therefore, separate repeated measures ANOVAs for the two levels of drug were performed. In the group treated with SCOP, the within-subject effect of stimulus duration on percentage omissions was not different per level of dose (no stimulus duration×dose

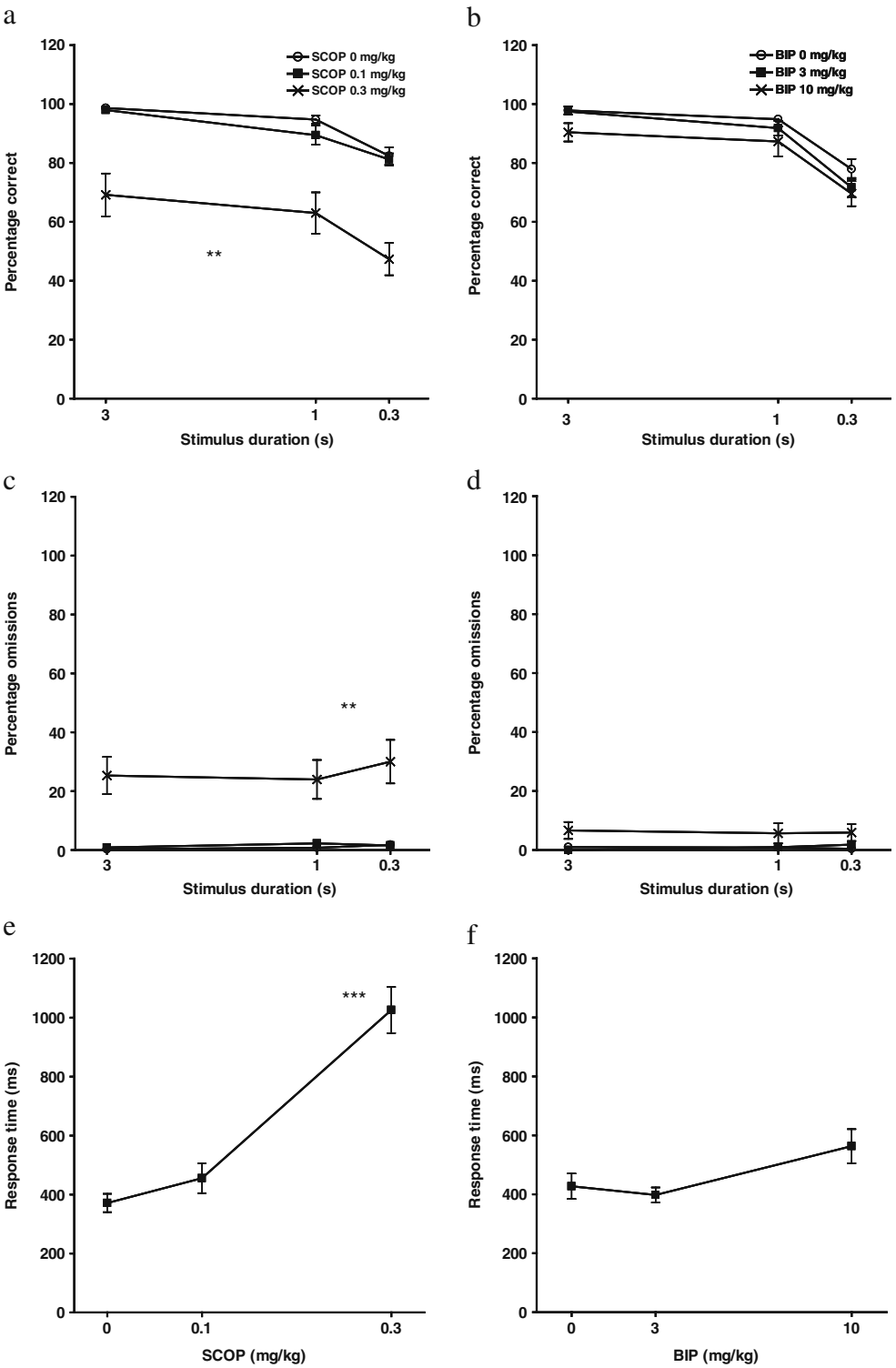

Fig. 3 The effects of SCOP (0.1, 0.3 mg/kg, IP) and BIP (3, 10 mg/kg, IP) on performance measures in the attention task. **a**, **b** Percentage correct responses. SCOP decreased accuracy independent of stimulus duration at a dose of 0.3 mg/kg. BIP did not have an effect on accuracy. **c**, **d** Percentage omissions. SCOP increased response omissions independent of stimulus duration at a dose of 0.3 mg/kg. BIP did not have an effect on response omissions. **e**, **f** Response time. SCOP slowed sensorimotor responding at a dose of 0.3 mg/kg. BIP did not have an effect on sensorimotor responding. Data represent mean (+SEM). *Asterisks* indicate differences from vehicle condition (**P<0.01; ***P<0.001)

interaction effect; $F(4, 36)=0.95$, n.s.; see Fig. 3c). There was no change in percentage omissions with shorter stimulus durations (no main effect of stimulus duration; $F(2, 18)=1.70$, n.s.). SCOP enhanced percentage omissions in the attention task (main effect of dose; $F(2, 18)=15.66$; $P<0.001$). Post hoc analysis showed that at a dose of 0.3 mg/kg SCOP augmented percentage omissions as compared with the vehicle condition ($P<0.01$). In the group treated with BIP, the within-subject effect of stimulus duration on percentage omissions was not different per level of dose (no stimulus duration×dose interaction effect; $F(4, 32)=0.61$, n.s.; see Fig. 3d). Moreover, shorter stimulus durations

had no effect on percentage omissions (no main effect of stimulus duration; $F(2, 16)=0.01$, n.s.). BIP treatment did not affect the measure percentage omissions (no main effect of dose; $F(2, 16)=3.31$, n.s.).

Figure 3e, f shows the effects of SCOP and BIP on response time in the attention task. The within-subject effect of dose on response time was found to vary per level of drug (dose×drug interaction effect; $F(2, 34)=5.59$; $P<0.01$). Therefore, separate repeated measures ANOVAs for the two levels of drug were performed. In the group treated with SCOP, response time was slowed (main effect of dose; $F(2, 18)=27.74$; $P<0.001$; see Fig. 3e). Post hoc analysis showed an effect of the 0.3 mg/kg dose ($P<0.001$). In the BIP group, response time was also significantly changed (main effect of dose; $F(2, 16)=4.28$; $P<0.05$; see Fig. 3f). However, post hoc analysis revealed no differences between vehicle and dose conditions.

Figure 4a, b shows the effects of SCOP and BIP on SI in the attention task. In the mixed model ANOVA, the within-subject effect of dose on SI did not vary per level of drug and stimulus duration (no dose×drug×stimulus duration interaction effect; $F(4, 68)=1.34$, n.s.). However, the within-subject effect of dose on SI did vary per level of drug (dose×drug interaction effect; $F(2, 34)=6.30$; $P<0.01$). The within-subject effect of stimulus duration on SI was also different per level of drug (stimulus duration×drug interaction effect; $F(2, 34)=4.63$; $P<0.05$). Therefore, separate repeated measures ANOVAs for the two levels of drug were performed. In the group treated with SCOP, the within-subject effect of stimulus duration on SI varied per level of dose (stimulus duration×dose interaction effect; $F(4, 36)=2.82$; $P<0.05$; see Fig. 4a). Hence, separate repeated measures ANOVAs were performed per level of stimulus duration. Post hoc analyses showed that SCOP reduced SI at al stimulus duration conditions. In the group treated with BIP, the within-subject effect of stimulus duration on SI was not different per level of dose (no stimulus duration×dose interaction effect; $F(4, 32)=0.12$, n.s.; see Fig. 4b). Shorter stimulus durations reduced SI (main effect of stimulus duration; $F(2, 16)=151.65$; $P<0.001$). BIP treatment did not affect the measure SI (no main effect of dose; $F(2, 16)=1.15$, n.s.).

Figure 4c, d shows the effects of SCOP and BIP on index *Y* in the attention task. In the mixed model ANOVA, the within-subject effect of dose on response bias did not vary per level of drug and stimulus duration (no dose×drug×stimulus duration interaction effect; $F(4, 68)=0.68$, n.s.). The within-subject effect of stimulus duration on index *Y* was also not different per level of drug (no stimulus duration×drug interaction effect; $F(2, 34)=0.11$, n.s.). The within-subject effect of dose on response bias did not vary per level of drug (no dose×drug interaction effect; $F(2, 34)=1.53$, n.s.). Index *Y* was differentially affected by dose (main effect of dose; $F(2, 34)=4.00$; $P<0.05$). Post hoc analysis showed that the highest dose conditions of SCOP and BIP augmented index *Y* as compared with the vehicle condition ($P<0.05$). However, the between-subject analysis of drug showed that SCOP and BIP did not differentially affect index *Y* (no main effect of drug; $F(1, 17)=3.13$, n.s.). Because we sought to determine which drug was responsible for the main effect of dose in the mixed model analysis, we did separate repeated measures ANOVAs per level of drug. In the group treated with SCOP, the within-subject effect of stimulus duration on index *Y* was not different per level of dose (no stimulus duration×dose interaction effect; $F(4, 36)=0.32$, n.s.; see Fig. 4c). There was a change in index *Y* with shorter stimulus durations (main effect of stimulus duration; $F(2, 18)=12.40$; $P<0.001$). SCOP did not affect the index *Y* measure (no main effect of dose; $F(2, 18)=3.35$, n.s.). In the group treated with BIP, the within-subject effect of stimulus duration on SI was not different per level of dose (no stimulus duration×dose interaction effect; $F(4, 32)=1.79$, n.s.; see Fig. 4d). Shorter stimulus durations reduced SI (main effect of stimulus duration; $F(2, 16)=15.22$; $P<0.001$). BIP treatment did not affect the measure SI (no main effect of dose; $F(2, 16)=1.48$, n.s.).

Delayed nonmatching to position

Figure 5a, b shows the effects of SCOP and BIP on percentage correct in the DNMTP task. In the mixed model ANOVA on percentage correct, the dose effect varied per level of delay and drug (dose×delay×drug interaction effect; $F(8, 144)=3.69$; $P<0.01$). Therefore, separate repeated measures ANOVAs for the two levels of drug were performed. In the group treated with SCOP, the within-subject effect of delay on percentage correct was different per level of dose (delay×dose interaction effect; $F(8, 72)=3.84$; $P<0.01$; see Fig. 5a). Hence, additional repeated measures ANOVAs were performed per level of delay. SCOP affected percentage correct at the 0-, 2-, 4-, and 8-s delay conditions (main effect of dose; F's$(2, 18)>9.23$; $P<0.01$). Post hoc analyses showed that a dose of 0.3 mg/kg was different from the vehicle condition. In addition, a dose of 0.1 mg/kg also affected accuracy performance at the 8-s delay condition. SCOP had no effect on percentage correct at 16-s delay condition (no main effect of dose; $F(2, 18)=0.31$, n.s.).

In the group treated with BIP, the within-subject effect of delay on percentage correct was different per level of dose (delay×dose interaction effect; $F(8, 72)=2.30$; $P<0.05$; see Fig. 5b). Hence, separate repeated measures ANOVAs were performed per level of delay. BIP did not affect percentage correct at the 0- and 16-s delay condition (no main effect of

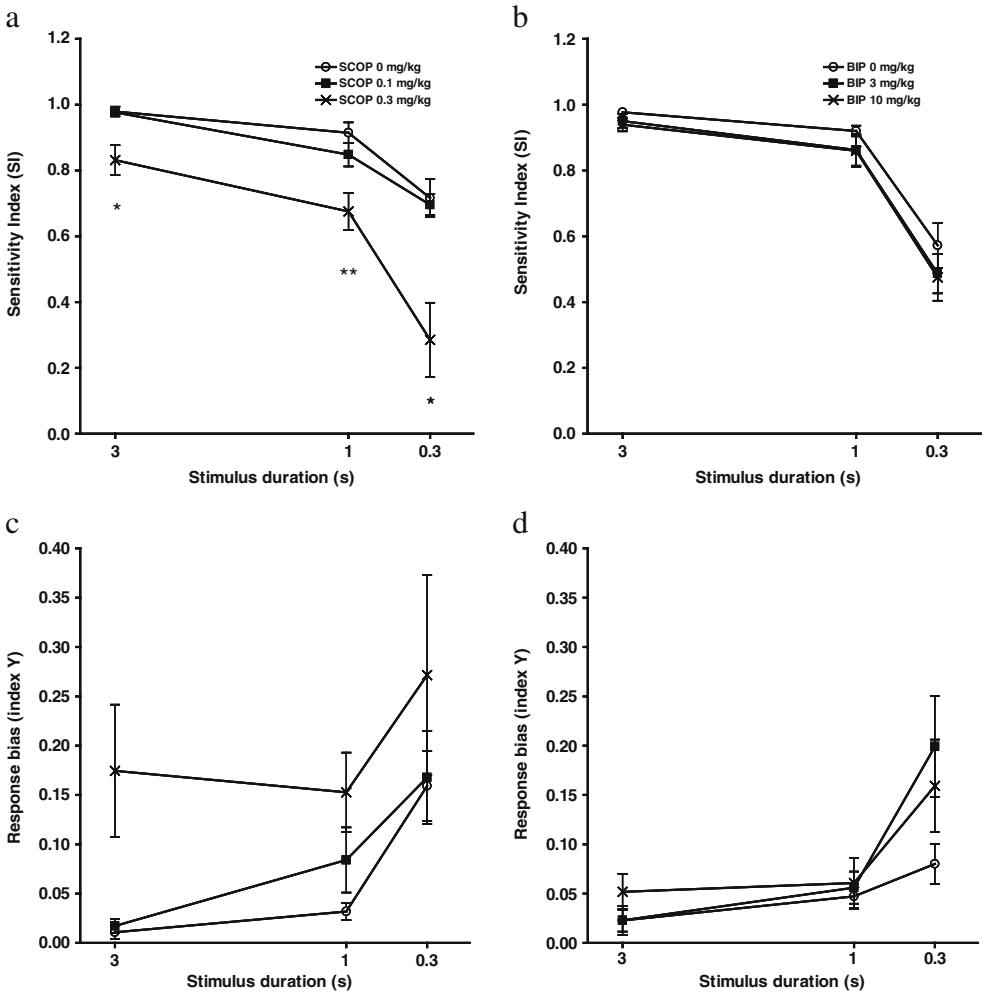

Fig. 4 The effects of SCOP (0.1, 0.3 mg/kg, IP) and BIP (3, 10 mg/kg, IP) on signal detection theory measures in the attention task. **a**, **b** Sensitivity Index (SI). SCOP decreased discriminability dependent of stimulus duration at a dose of 0.3 mg/kg. BIP did not have an effect on discriminability. **c**, **d** Index *Y*. Neither SCOP nor BIP had an effect on response bias. Data represent mean (+SEM). *Asterisks* indicate differences from vehicle condition (*P<0.05; **P<0.01)

dose; F's(2, 18)<2.95, n.s.). However, BIP impaired percentage correct in trials with a 2-, 4-, and 8-s delay (main effect of dose; F's(2, 18)>5.84; P<0.05). Separate post hoc analyses demonstrated that the 3 mg/kg dose decreased percentage correct as compared with the vehicle condition.

Figure 5c, d shows the effects of SCOP and BIP on response time in the DNMTP task. The within-subject effect of dose on response time did not vary per level of drug (no dose×drug interaction effect; F(2, 36)=2.06, n.s.). There was no effect of dose (no main effect of dose; F(2, 36)=1.06, n.s.). SCOP and BIP did not differentially affect response time (no main effect of drug; F(1, 18)=1.44, n.s.).

Figure 6a, b shows the effects of SCOP and BIP on SI in the DNMTP task. In the mixed model ANOVA on SI, the dose effect varied per level of delay and drug (dose×delay×drug interaction effect; F(8, 144)=3.68; P<0.01). Therefore, separate repeated measures ANOVAs for the two levels of drug were performed. In the group treated with SCOP, the within-subject effect of delay on SI was different per level of dose (delay×dose interaction effect; F(8, 72)=3.84; P<0.01; see Fig. 6a). Hence, additional repeated measures ANOVAs were performed per level of delay. In the 0-, 2-, 4-, and 8-s delay trials a main effect of dose was found (F's(2, 18)>9.23; P<0.01). Separate post hoc analyses showed that at a dose of 0.3 mg/kg SCOP reduced SI as compared with the vehicle condition. In addition a dose of 0.1 mg/kg also affected performance at the 8-s delay condition. SCOP had no effect on SI in trials which used a 16-s delay (no main effect of dose; F(2, 18)=0.31, n.s.).

In the group treated with BIP, the within-subject effect of delay on SI was different per level of dose (delay×dose interaction effect; F(8, 72)=2.30; P<0.05; see Fig. 6b). Hence, separate repeated measures ANOVAs were performed per level of delay. In trials with a 0- or 16-s delay BIP did not affect SI (no main effect of dose; F's(2, 18)<2.95, n.s.). BIP did influence SI in trials a 2-, 4-, and 8-s delay (main effect of dose; F's(2, 18)>5.84; P<0.05). Post hoc analysis revealed that the 3 mg/kg dose decreased SI as compared with the vehicle condition (P<0.01).

Figure 6c, d shows the effects of SCOP and BIP on index *Y* in the DNMTP task. In the mixed model ANOVA, the within-subject effect of dose on response bias did not vary per level of drug and delay (no dose×drug×delay interaction effect; F(8, 136)=1.13, n.s.). The within-subject

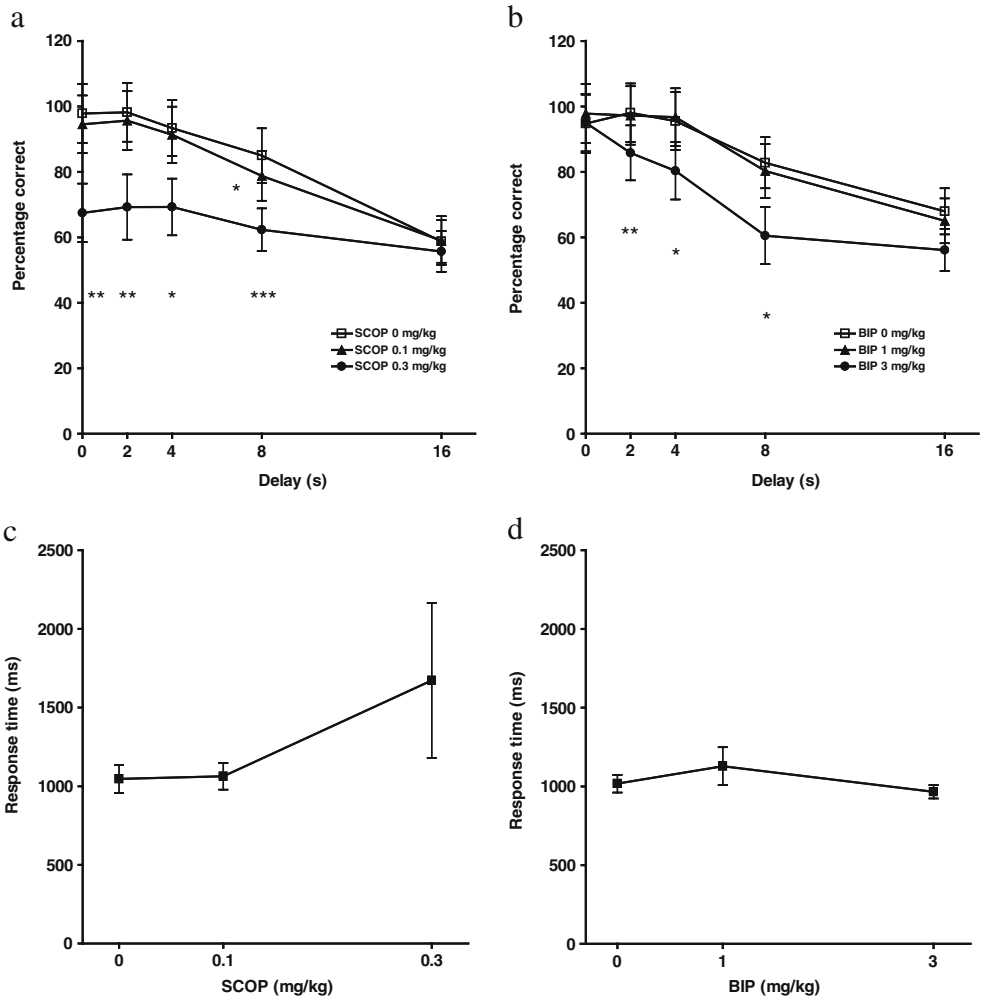

Fig. 5 The effects of SCOP (0.1, 0.3 mg/kg, IP) and BIP (1, 3 mg/kg, IP) on performance measures in delayed non-matching to position. **a**, **b** Percentage correct responses. SCOP decreased accuracy delay-dependently at a dose of 0.1 and 0.3 mg/kg. BIP reduced accuracy delay-dependently at a dose of 3 mg/kg. **c**, **d** Response time. Neither SCOP nor BIP had an effect on sensorimotor responding. Data represent mean (+SEM). *Asterisks* indicate differences from vehicle condition (*$P<0.05$; **$P<0.01$; ***$P<0.001$)

effect of delay on index *Y* was also not different per level of drug (no delay×drug interaction effect; $F(4, 68)=0.43$, n.s.). Moreover, the within-subject effect of dose on response bias did not vary per level of drug (no dose×drug interaction effect; $F(2, 34)=3.06$, n.s.). The within-subject effect of dose on index *Y* was also not different per level of delay (no dose×delay interaction effect; $F(8, 136)=1.78$, n.s.). Index *Y* was differentially affected by dose (main effect of dose; $F(2, 34)=22.24$; $P<0.001$). The between-subject analysis of drug showed that SCOP and BIP did differentially affect index *Y* (main effect of drug; $F(1, 17)=4.49$; $P<0.05$). Therefore, separate repeated measures ANOVAs for the two levels of drug were performed. In the group treated with SCOP, the within-subject effect of delay on response bias was not different per level of dose (no delay×dose interaction effect; $F(8, 64)=1.67$, n.s.; see Fig. 6c). Index *Y* increased with longer delays (main effect of delay; $F(4, 32)=12.28$; $P<0.001$). SCOP enhanced response bias in the DNMTP task (main effect of dose; $F(2, 16)=11.62$; $P<0.01$). Post hoc analysis showed that at a dose of 0.3 mg/kg SCOP augmented index *Y* as compared with the vehicle condition ($P<0.01$). In the group treated with BIP, the within-subject effect of delay on index *Y* was not different per level of dose (no delay×dose interaction effect; $F(8, 72)=1.29$, n.s.; see Fig. 6d). Moreover, with longer delays response bias was increased (main effect of delay; $F(4, 36)=18.92$; $P<0.001$). BIP was shown to increase response bias in the DNMTP task (main effect of dose; $F(2, 18)=12.55$; $P<0.001$). Post hoc analysis showed that at a dose of 3 mg/kg BIP augmented index *Y* as compared with the vehicle condition ($P<0.01$).

Discussion

The main objective of the current study was to compare the effects of the nonselective muscarinic antagonist SCOP and the m1 antagonist BIP after systemic injections on different aspects of operant behavior: sensorimotor responding (FR5), food motivation (PR10), attention and short-term memory (DNMTP). The direct comparison of both drugs allowed evaluation with respect to the usability of BIP, as opposed to SCOP, as a suitable model of cholinergic memory dysfunction. Since BIP is relatively selective for m1 receptors which can be found predominantly in brain

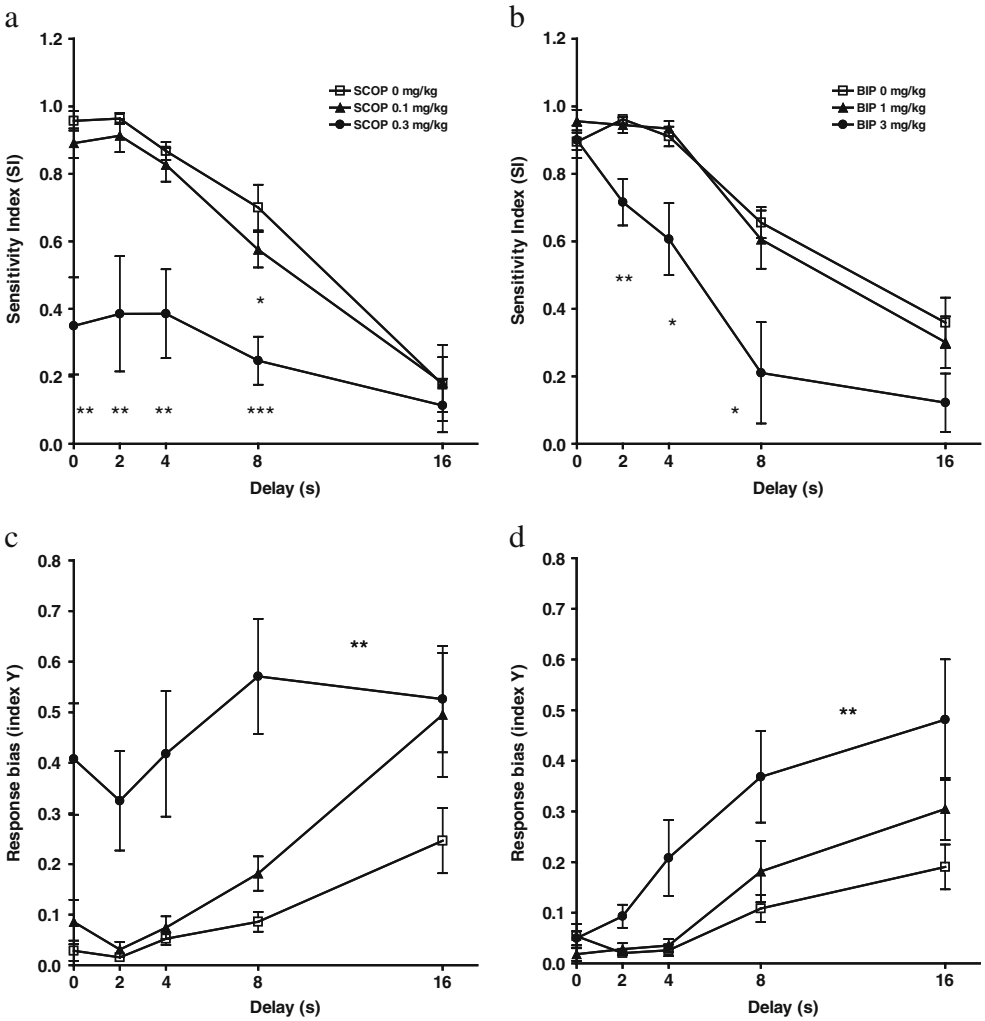

Fig. 6 The effects of SCOP (0.1, 0.3 mg/kg, IP) and BIP (1, 3 mg/kg, IP) on signal detection measures in delayed non-matching to position. **a**, **b** Sensitivity Index (SI). SCOP decreased discriminability delay-dependently at a dose of 0.1 and 0.3 mg/kg. BIP reduced discriminability delay-dependently at a dose of 3 mg/kg. **c**, **d** Index *Y*. SCOP decreased response bias delay-independently at a dose of 0.3 mg/kg. BIP reduced response bias delay-independently at a dose of 3 mg/kg. Data represent mean (+SEM). *Asterisks* indicate differences from vehicle condition (**P*<0.05; ***P*<0.01; ****P*<0.001)

areas involved in learning and memory (Caulfield 1993; Volpicelli and Levey 2004), we expected also a more selective effect of BIP on cognition and behavioral performance. In Table 1 an overview is given of the effects of both drugs on the various behavioral measures. A wide range of behavioral impairments were found after SCOP; cognitive and peripheral effects were not dissociable on basis of dose conditions. Moreover, performance deficits in the short-term memory task were of a nonmnemonic nature. In contrast, BIP more selectively impaired DNMTP performance at a dose of 3 mg/kg, at which no peripheral effects were found; sensorimotor responding was slowed after the 10 mg/kg dose.

Of note, some caution should be taken with respect to the selectivity of muscarinic (ant)agonists. Due to the highly conserved nature of the orthosteric binding site amongst muscarinic receptors, drugs that target these can be characterized as possessing relative rather than absolute receptor subtype selectivity. These issues should be borne in mind when trying to attribute effects of these drugs to specific muscarinic receptor subtypes. BIP for instance has about tenfold higher affinity for m1 as compared with m2–m5 receptors (see Bolden et al. 1992; Katayama et al. 1990). The pharmacokinetic characteristics of BIP are quite favorable; cerebellar levels after a dose of 3.2 mg/kg (IV) have been reported to be about ten times higher as plasma levels (see Nakashima et al. 1993; Syvälahti et al. 1988; Yokogawa et al. 1990; Yokogawa et al. 1992). Regardless, BIP is currently the drug of choice for making a direct comparison with SCOP, particularly as it is approved for use in humans and therefore suitable for translational research. Some other drugs that target the muscarinic m1 receptor cannot be used in systemic injections because they do not cross the blood–brain barrier (e.g., pirenzepine).

Sensorimotor responding

Both SCOP and BIP were found to slow sensorimotor responding on a FR5 schedule of reinforcement, although at a different dose (0.3 and 1 mg/kg for SCOP and 10 mg/kg for BIP, see Fig. 1). Although both SCOP and BIP increased inter-response time in the FR5 task it should be

Table 1 An overview of the effects of SCOP and BIP on sensorimotor responding, food motivation, attention, and short-term memory

	Drug	
Behavioral task	SCOP	BIP
FR5	Inter-response time ↑ (0.3, 1)	Inter-response time ↑ (10)
PR10	Breakpoint ↓ (1)	Breakpoint =
	Inter-response time =	Inter-response time =
AT	Percentage correct ↓ (0.3, DI)	Percentage correct =
	Percentage omissions ↑ (0.3, DI)	Percentage omissions =
	Response time ↑ (0.3)	Response time =
	Sensitivity index ↓ (0.3, DD)	Sensitivity index =
	Index *Y* =	Index *Y* =
DNMTP	Percentage correct ↓ (0.1, 0.3, DD)	Percentage correct ↓ (3, DD)
	Response time =	Response time =
	Sensitivity index ↓ (0.1, 0.3, DD)	Sensitivity index ↓ (3, DD)
	Index *Y* ↑ (0.3, DI)	Index *Y* ↑ (3, DI)

Doses are given in milligrams per kilogram, IP

Abbreviations: *FR5* fixed ratio 5, *PR10* progressive ratio 10, *AT* attention task, *DNMTP* delayed nonmatching to position, *SCOP* scopolamine hydrobromide, *BIP* biperiden, *DD* delay-dependent effect, *DI* delay-independent effect, *equal sign* no change, *upward arrow* increase, *downward arrow* decrease

noted that the effects of BIP were smaller as compared with SCOP. BIP, at a dose of 10 mg/kg, slowed FR5 responses by 26% as compared with the vehicle condition. SCOP, at a dose of 0.3 and 1 mg/kg, increased inter-response time by about 32% and 298%, respectively. In FR tasks, SCOP has generally been found to decrease lever presses independent of reward type (dry vs. wet, Hodges et al. 2009). However, the minimal effective dose reported in these studies does show quite some variability (0.005–1.0 mg/kg IP, Hodges et al. 2009; Pradhan and Roth 1968). In a brightness discrimination task, BIP (0.25–2 mg/kg, SC) was found to reduce the rate of reinforcement (Liu 1996). Furthermore, intracerebroventricular infusion of the m1 antagonist pirenzepine (10, 30 μg in 2.5 μL) increased the sample latency in a DNMTP task (Aura et al. 1997), although a reduction in response latency has also been reported (Andrews et al. 1994).

These results suggest that the effect of systemic administration of SCOP and BIP on sensorimotor responding is at least partially mediated by the m1 receptor. However, from the present data it cannot be deduced whether this change in sensorimotor responding is caused by central or peripheral m1 blockade, or both. For instance, in the periphery m1 receptors have been found in abundance in rat sympathetic ganglia such as the superior cervical ganglion (Caulfield and Birdsall 1998). However, the effects of SCOP and BIP on sensorimotor responding could also result from an interaction between m1 and striatal dopaminergic signaling (De Klippel et al. 1993; Gerber et al. 2001).

Food motivation

SCOP (1 mg/kg) was found to decrease food motivation and slow sensorimotor responding on a PR10 schedule, whereas BIP (3 and 10 mg/kg) did not have an effect on these measures (see Fig. 2). This is in accordance with studies in monkeys performing a PR schedule where SCOP reduced the number of obtained reinforcements (Spinelli et al. 2006; Taffe et al. 1999). Food and water intake in rats was found to be diminished after SCOP administration (Hodges et al. 2009; Pradhan and Roth 1968). To the best of our knowledge, neither BIP, nor any other m1 antagonists have been tested in paradigms assessing food motivation and/or free feeding behavior. Although it is possible that a higher dose of BIP would have yielded a reduction in motivation, this dose is comparable to dose conditions used in other behavioral studies (Jones and Shannon 2000; Kimura et al. 1999; Liu 1996; Myers et al. 2002; Myhrer et al. 2008; Roldán et al. 1997; Sipos et al. 1999; 2001).

Again, it cannot be decisively determined whether the decrement in motivation for food after administration of SCOP is a central or a peripheral effect. SCOP has been known to induce “dry mouth” (Hodges et al. 2009), which might affect the palatability and thus the hedonic impact of dry food rewards. Of note, when using a liquid reward no peripheral effect of SCOP has been found in FR5 and DNMTP paradigms (Hodges et al. 2009). Nevertheless, a decrease in “liking” dry food rewards after systemic administration of SCOP could interfere with behavioral performance. Particularly the m3 (Dai et al. 1991; Shida et al. 1993) but also the m1 and m5 receptors have been implicated in rat salivary responses (Flynn et al. 1997; Shannon et al. 1994; Tobin et al. 2002). Thus, according to the literature BIP is capable of interfering with salivation to some extent. However, the current data suggest that any reductions in salivation after BIP doses of 10 mg/kg and lower are not sufficient to interfere with food motivation. These findings are in contrast with those of SCOP, which is likely to more fully block muscarinic receptor subtypes in rat salivary glands and to profoundly affect food motivation. A central effect of SCOP might also interfere with

incentive-driven behaviors (such as PR performance). Centrally infused SCOP in rat nucleus accumbens has been found to reduce sucrose consumption (1 or 10 μg/side) and breakpoint (5.0 μg/side) in a PR paradigm (Pratt and Kelley 2004). Furthermore, muscarinic receptors appear to be implicated in reward-driven motivational behaviors via excitatory interactions with dopamine in the nucleus accumbens and ventral tegmental area (Forster et al. 2001; Yeomans and Baptista 1997).

Attention

SCOP affected performance in the attention task at all stimulus conditions (see Figs. 3, 4). At a dose of 0.3 mg/kg, SCOP decreased percentage correct, increased percentage omissions and response time independent of the duration of the stimulus. Discriminability (SI) was also reduced after the 0.3 mg/kg dose; however, the effect of SCOP was dependent on stimulus duration. Response bias (index *Y*) was unaffected after SCOP or BIP (3 and 10 mg/kg). BIP also did not affect any of the other measures in the attention task. In attentional paradigms such as the five-choice serial reaction time task, SCOP has been reported to disrupt visuospatial sustained attention at doses of 0.02 mg/kg and higher (Callahan et al. 1993; Cheal 1981; Hodges et al. 2009; Hoff et al. 2007; Humby et al. 1999; Leblond et al. 2002; Spinelli et al. 2006); however, behavioral effects of SCOP on attentional accuracy are not reported consistently (Andrews et al. 1992; Doty et al. 2003; Leaton and Kreindler 1972). Moreover, SCOP has been shown to influence general noncognitive performance measures, such as response latency and number of missed trials (Andrews et al. 1992; Bushnell et al. 1997; Drinkenburg et al. 1995). As SCOP also disrupted performance in the FR5 and PR10 tasks, its effect on attention could (partially) be caused by deficits in sensorimotor responding and/or food motivation. Moreover, it is unlikely that m1 receptor blockade underlies the attentional impairment, as BIP had no effect on this task. To the best of our knowledge, effects of m1 antagonists have not been assessed in attentional paradigms before. Further studies are required in order to provide more support for the lack of a role of m1 and potentially other muscarinic receptors in attention.

Short-term memory

Similar to its effects on the attention task, SCOP impaired various performance measures in the DNMTP task (see Figs. 5, 6). SCOP decreased percentage correct and discriminability (SI) in a delay-dependent manner, and increased response bias (index *Y*) delay-independently at a dose of 0.3 mg/kg. SCOP already affected DNMTP performance at the shortest delay(s), whereas it failed to disrupt performance in trials with the longest delay. The latter is likely due to a floor effect; i.e., accuracy of the animals in the 16-s delay trials was already at around chance level (50% correct) even in the vehicle condition, and therefore further impairment due to cholinergic blockade could not be induced. The different delay intervals between sample and choice phase are presumed to produce a temporal performance gradient with longer retention intervals yielding poorer DNMTP performance as short-term memory functions are taxed in an increasing manner. Thus, the disruption of DNMTP performance at the zero delay indicates an effect of SCOP on other (non)cognitive processes rather than just short-term memory. As the current study demonstrates, the effects of SCOP (0.3 mg/kg) on sensorimotor responding, food motivation and/or attention could (at least partially) underlie deficits in DNMTP performance.

The majority of studies using delayed (non)matching procedures have reported a delay-independent impairment after relatively low doses (e.g., 0.05, 0.075, and 0.1 mg/kg IP, Herremans et al. 1995; 0.1 mg/kg IP, Hodges et al. 2009), which again suggests that SCOP does not specifically affect short-term memory functions – although some articles have challenged this finding (Estape and Steckler 2002; Ruotsalainen et al. 1998; Santi and Weise 1995; Stanhope et al. 1995). Furthermore, in most studies using systemic injections SCOP also affected measures of responding; it increased number of omissions, decreased number of completed trials and increased response latency (Estape and Steckler 2002; Kirkby et al. 1995). Central administration of SCOP in the medial prefrontal cortex (Dunnett et al. 1990; Herremans et al. 1997; Herremans et al. 1996), prelimbic cortex (Granon and Poucet 1995), and hippocampus (Robinson and Mao 1997) has been shown to yield a delay-independent reduction of DNMTP response accuracy (but see Broersen et al. 1994; Broersen et al. 1995; Dunnett et al. 1990; Granon et al. 1995) and increases in number of omissions (Robinson and Mao 1997).

It is likely that DNMTP deficits produced by SCOP can be partially attributed to m1 blockade, which is in line with the results reported by Bymaster et al. (1993). BIP (3 mg/kg) was found to decrease percentage correct and discriminability (SI) in a delay-dependent manner, and increase response bias (index *Y*) delay-independently. BIP did not influence response time at the doses used in the DNMTP (1 and 3 mg/kg, IP) which is in line with our findings in the FR5 task. As is shown in Figs. 5 and 6, BIP did not affect DNMTP performance at the zero delay. However, as the delay interval increased, BIP increasingly impaired accuracy performance as compared with the vehicle condition. Thus, the disruption of DNMTP performance at longer delays but not the shortest delay indicates a genuine effect of BIP on short-term memory functions rather than other

(non)cognitive processes. These effects are unlikely to be caused by deficits in sensorimotor responding, food motivation or attention, as BIP (at a dose of 3 mg/kg) did not affect performance on the FR5 or PR10 schedule of reinforcement or in the attention task. Of note, the lack of an effect of BIP at the 16-s delay is again likely due to a floor effect. Taken together, these findings suggest a role for the m1 receptor in mediating short-term memory functions. This would implicate selective m1 antagonists such as BIP as a promising alternative instead of the gold standard drug SCOP as a tool for inducing cholinergic mnemonic impairments in animals.

Effects of systemic administration of BIP on DNMTP performance have not been assessed previously; however, m1 antagonists have been found to affect performance in a variety of other behavioral tasks which measure (short-term) memory. For instance, after systemic injections deficits have been reported in passive avoidance tasks (Fornari et al. 2000; Kimura et al. 1999; Kramer-Soares et al. 2006; Roldán et al. 1997), contextual fear conditioning (Kramer-Soares et al. 2006; but see Sheffler et al. 2009), spatial alternation (Bymaster et al. 1993), and object recognition (Myhrer et al. 2004; 2008). Furthermore, m1 agonists have been shown to improve DNMTP performance in animals which were cognitively impaired after cholinergic lesioning (McDonald et al. 1998), SCOP administration or aging (Bartholomeo et al. 2000), which suggests that enhanced m1 signaling can be sufficient in order to reverse memory deficits. Infusion of the muscarinic m1 antagonist pirenzepine (35 μg in 0.5 μL/side) in the dorsal hippocampus impaired accuracy performance on a DNMTP task (Messer et al. 1990; Messer et al. 1987); this implicates the importance of m1 receptor signaling in the hippocampus for accurate DNMTP responding.

Of note, there are some indications that the m1 receptor might also be involved in reversal learning (McCool et al. 2008; Tzavos et al. 2004) and anxiety (Wall et al. 2001). In addition, muscarinic (m1) blockade in nonhippocampal brain regions might also be responsible for short-term memory effects; for instance, intact performance on non-matching tasks seems to also require the prefrontal, entorhinal, and perirhinal cortices (Otto and Eichenbaum 1992) Thus, our study does not exclude the involvement of muscarinic receptor subtypes other than m1 and brain regions other than the septo-hippocampal system in memory functions, nor the engagement of the m1 receptor in other cognitive processes besides memory (see for instance Araya et al. 2006; Carey et al. 2001; Daniel and Dohanich 2001; McCool et al. 2008; Messer and Miller 1988; Poulin et al. 2010; Power et al. 2003; Quirion et al. 1995; Tzavos et al. 2004; Wall et al. 2001; Wess 2004).

In fact, the manner in which m1 receptors affect memory processes is still under investigation; one possibility is the modulation of glutamatergic neurotransmission and/or synaptic plasticity (see Caulfield 1993; Hasselmo 1999; 2006). Muscarinic m1 receptors couple to Gq-proteins which activate several signaling cascades via phospholipase (PL)C (Caulfield 1993; Jones 1993; Liu et al. 2006), which can ultimately influence Ca^{2+} and K^{+} currents (Liu et al. 2006), raise cyclic AMP levels (Jones 1993), and can stimulate other receptor systems such as glutamatergic *N-methyl*-D-aspartate (NMDA) receptor currents produced by hippocampal CA1 pyramidal neurons (Calabresi et al. 1998; Ma et al. 2009; Marino et al. 1998). Moreover, m1 receptors and NR1a NMDA receptor subunits were found to be colocalized at glutamatergic synapses, suggestive of a direct interaction between the two receptor systems. A link between m1 receptor signaling and long-term potentiation (LTP), a mechanism which is thought to underlie learning and memory processes, has also been put forward (Boddeke et al. 1992; Burgard and Sarvey 1990; Calabresi et al. 1999; Doralp and Leung 2008; Kamsler et al. 2010; Ovsepian et al. 2004; Shinoe et al. 2005). For instance, it has been shown that muscarinic agonists and antagonists which act preferentially on the m1 receptor are able to facilitate or prevent the induction of LTP in rat dentate gyrus (Burgard and Sarvey 1990), CA1 (Boddeke et al. 1992; Doralp and Leung 2008; Ovsepian et al. 2004), and striatum (Calabresi et al. 1999), respectively.

Future studies on the role of muscarinic receptors should focus on determining whether there exists some degree of dissociation between muscarinic receptor subtypes in terms of their involvement in memory (or other cognitive functions) as is reflected by their differential distribution in the brain (e.g., Rouse and Levey 1996). It is likely that particular muscarinic subtypes are only important for a restricted (set of) cognitive subdomain(s); e.g., hippocampal m1 receptors are important for working but not reference memory (Ohno et al. 1994). Furthermore, it is imperative that the manner in which m1 receptors influence memory processes is more extensively investigated. Information on the exact signaling cascade(s) downstream of the muscarinic m1 receptor that are responsible for its effects on memory could lead to interesting implications for the development of novel treatments for disorders in which memory is impaired, such as Alzheimer's disease or schizophrenia. Lastly, additonal behavioral validation is required to firmly establish the usability of m1 antagonists instead of the gold standard SCOP for producing cholinergic amnesia in healthy animals and human participants.

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