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ORIGINAL INVESTIGATION

The role of the cholinergic system in the signal attenuation rat model of obsessive-compulsive disorder

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Abstract
Rationale In comparison to studies of the involvement of the serotonergic, dopaminergic, and glutamatergic systems in the pathophysiology of obsessive–compulsive disorder (OCD), research on the involvement of the cholinergic system in this disorder has remained sparse.
Objectives The aim of this study was to test the role of the cholinergic system in compulsive behavior using the signal attenuation rat model of OCD. In this model, "compulsive" behavior is induced by attenuating a signal indicating that a lever-press response was effective in producing food.
Methods The acetylcholinesterase inhibitor physostigmine (0.05, 0.10, and 0.15 mg/kg), the nicotinic agonist nicotine (0.03, 0.06, 0.10, 0.30, 0.60, and 1.00 mg/kg), the nicotinic antagonist mecamylamine (1, 3, 5, and 8 mg/kg), the muscarinic agonist oxotremorine (0.0075, 0.0150, and 0.0300 mg/kg), and the muscarinic antagonist scopolamine (0.15, 0.50, 1.00, and 1.50 mg/kg) were acutely administered to rats just before assessing their lever-press responding following signal attenuation (experiments 1, 3, 5, 7, and 9, respectively). Because the effects of signal attenuation are assessed under extinction conditions, drug doses that were effective in the above experiments were also tested in an extinction session of lever-press responding that was not preceded by signal attenuation (experiments 2, 4, 6, 8, and 10).
Results Acute systemic administration of the cholinergic agents did not exert a selective anti- or pro-compulsive effect in the signal attenuation model.
Conclusions Acetylcholine does not seem to play a role in the signal attenuation rat model of OCD.

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Introduction

Obsessive–compulsive disorder (OCD) is a psychiatric disorder with a lifetime prevalence of 1–3 % (Menzies et al. 2008; Sasson et al. 1997), characterized by recurrent, intrusive, and unwanted thoughts (obsessions) and/or repetitive ritualistic behaviors (compulsions) (American Psychiatric Association 1994). Although the etiology of OCD is unknown, the prevailing view is that its pathophysiology involves a dysfunction of the serotonergic, dopaminergic, and glutamatergic systems (Aouizerate et al. 2005; Denys et al. 2004; Pittenger et al. 2006).

There is some evidence suggesting that the cholinergic system may also be involved in the pathophysiology of OCD. Yet, current data do not converge to allow a hypothesis regarding the exact nature of cholinergic dysfunction in this disorder. Thus, magnetic resonance spectroscopy studies reported significantly higher levels of choline, the precursor of acetylcholine in the thalamus of OCD patients compared to healthy controls (Mohamed et al. 2007; Smith et al. 2003) and major depressive disorder patients (Smith et al. 2003). The growth hormone response in OCD patients following a challenge with the acetylcholine esterase inhibitor pyridostigmine was increased compared to healthy controls (Lucey et al. 1993), and although the incidence of smoking in OCD patients is lower compared with the general population (approximately 14 and 25 %, respectively Bejerot and Humble 1999), there are several reports of positive effects of nicotine on OCD patients (Lundberg et al. 2004; Pasquini et al. 2005; Salin-Pascual and Basanez-Villa 2003). Interestingly, attenuation of compulsive-like behavior by nicotine was reported in the quinpirole rat model of OCD (Tizabi et al. 2002).

The aim of the present study was to conduct a thorough assessment of the involvement of the cholinergic system in the signal attenuation rat model of OCD (for review of the model, see Albelda and Joel 2012; Joel 2006). This model was developed on the basis of the theoretical proposition that compulsive behaviors result from a deficit in the feedback associated with the performance of normal goal-directed response (Baxter 1999; Gray 1982; Malloy 1987; Otto 1992; Pitman 1987; Reed 1977; Szechtman and Woody 2004). In the model, a light and tone stimulus is established as a feedback cue that signals that a lever-press response was effective in producing food. The "signaling" property of the stimulus is then attenuated by repeatedly presenting the stimulus without food (without the rat emitting the lever-press response). In a subsequent extinction test, this manipulation (*signal attenuation*) leads to excessive lever pressing that is not accompanied by an attempt to collect a reward. This behavior has been named "compulsive" lever pressing because it may be analogous to the excessive and unreasonable behavior seen in OCD. Compulsive lever pressing is affected by manipulations of the orbitofrontal cortex and of the serotonergic, dopaminergic, and glutamatergic systems (Albelda et al. 2010; Flaisher-Grinberg et al. 2008; Joel and Avisar 2001; Joel and Doljansky 2003; Joel and Klavir 2006; Joel et al. 2004, 2005a, b), in line with evidence implicating these systems in OCD. Of particular importance for the present study, compulsive lever pressing is abolished by acute administration of the selective serotonin reuptake inhibitors (SSRIs) fluoxetine, paroxetine, and fluvoxamine, but not by the anxiolytic drug diazepam, the antipsychotic haloperidol, or the tricyclic antidepressant desipramine (Joel and Avisar 2001; Joel et al. 2004; Joel and Doljansky 2003), in accordance with the differential efficacy of these drugs in alleviating obsessions and compulsions in OCD patients (Dolberg et al. 1996; Piccinelli et al. 1995; Zohar et al. 1992). On the basis of these results, it has been suggested that the signal attenuation model may be used to screen drugs for anti-compulsive activity using acute administration regimen (Joel et al. 2004).

The present study therefore tested whether manipulations of the cholinergic system by acute systemic administration of cholinergic agonists and antagonists affect compulsive lever pressing. The following drugs were tested: the acetylcholinesterase inhibitor physostigmine, the nicotinic agonist nicotine, the nicotinic antagonist mecamylamine, the muscarinic agonist oxotremorine, and the muscarinic antagonist scopolamine. Since the effects of signal attenuation on rats' lever-press responding are assessed under extinction conditions and drug manipulations may affect behaviors typical to extinction (e.g., extinction burst), drug doses that were effective in the post-training signal attenuation (PTSA) procedure were also tested in an extinction session that was not preceded by signal attenuation (a procedure referred to as "regular extinction"). An anti- or pro-compulsive effect in the model is evidenced in a decrease or increase, respectively, in the number of excessive lever presses that are not followed by magazine entry in rats that underwent signal attenuation but not in rats that underwent regular extinction (for further exposition, see Joel 2006).

Methods

Subjects

Four hundred and ninety-three male Sprague–Dawley rats (Tel Aviv University, Israel), approximately 3–4 months old, were housed two or three to a cage under a reversed 12-h light–dark cycle (lights on 1900–0700 hours) and maintained on a 22-h food restriction schedule (food was provided in the home cage at least half an hour after the end of behavioral training), with water freely available. Rats were weighed twice a week to ensure that their body weight was not reduced to below 90 %. All experimental protocols were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University (see Table 1 for individual group *n*'s).

Apparatus and behavioral procedure

Behavioral testing was conducted in operant conditioning chambers (Campden Instruments, Loughborough, UK), housed in sound-attenuated boxes and equipped with a 3-W house light, a Sonalert module (model 80223) that could produce an 80-dB 2.8-kHz tone, and two retractable levers on either side of a food magazine (fitted with a 3-W magazine light), into which 45 mg Noyes precision food pellets (PMI Nutrition International, Indiana, United States) could be delivered. Access to the food magazine was through a hinged panel, the opening of which activated a microswitch. Equipment programming and data recording were computer controlled using the Animal Behavior Environment Test System software (Lafayette Instrument Company, Lafayette, IN, USA).

Prior to the beginning of the experiment, rats were handled for about 2 min daily for 5 days. On the last 3 days after handling, ~20 food pellets used as reinforcement for operant training were introduced into the home cages.

Post-training signal attenuation The post-training signal attenuation procedure included four stages. The organization of a trial in each of these stages is presented in Fig. 1.

Stage 1 Magazine training. On days 1–3, rats were trained to collect food pellets from the food magazine in the operant chamber, with the levers retracted. On

Table 1 Number of rats in each experiment and in each group

Experiment	Number of rats	Number of rats excluded[a]	Number of rats in each group
Physostigmine (0.05, 0.10, and 0.15 mg/kg) in PTSA	38	1	Vehicle–12
			0.05–12
			0.10–8
			0.15–5
Physostigmine (0.1 mg/kg) in PTSA and RE	48	1	PTSA vehicle–12
			RE vehicle–12
			PTSA drug–12
			RE drug–11
Nicotine (0.03, 0.06, 0.10, 0.30, 0.60, and 1.00 mg/kg) in PTSA	117	2	Vehicle–31
			0.03–9
			0.06–9
			0.10–10
			0.30–20
			0.60–20
			1.00–16
Nicotine (0.3 mg/kg) in PTSA and RE	48	0	PTSA vehicle–12
			RE vehicle–12
			PTSA drug–12
			RE drug–12
Mecamylamine (1, 3, and 5 mg/kg) in PTSA	48	3	Vehicle–11
			1–11
			3–12
			5–11
Mecamylamine (8 mg/kg) in PTSA and RE	42	1	PTSA vehicle–10
			RE vehicle–11
			PTSA drug–10
			RE drug–10
Oxotremorine (0.0075, 0.0150, and 0.0300 mg/kg) in PTSA	30	2	Vehicle–7
			0.0075–6
			0.0150–7
			0.0300–8
Oxotremorine (0.03 mg/kg) in PTSA and RE	31	1	PTSA vehicle–6
			RE vehicle–8
			PTSA drug–8
			RE drug–8
Scopolamine (0.15, 0.50, and 1.50 mg/kg) in PTSA	48	4	Vehicle–12
			0.15–11
			0.50–11
			1.50–10
Scopolamine (0.5 and 1.0 mg/kg) in PTSA and RE	60	2	PTSA vehicle–10
			RE vehicle–10
			PTSA, 0.5–9
			RE, 0.5–10
			PTSA, 1.0–10
			RE, 1.0–9

[a] Rats were excluded if their score on ELP-C and/or ELP-U was more than four standard deviations above their group mean

PTSA post-training signal attenuation, *RE* regular extinction

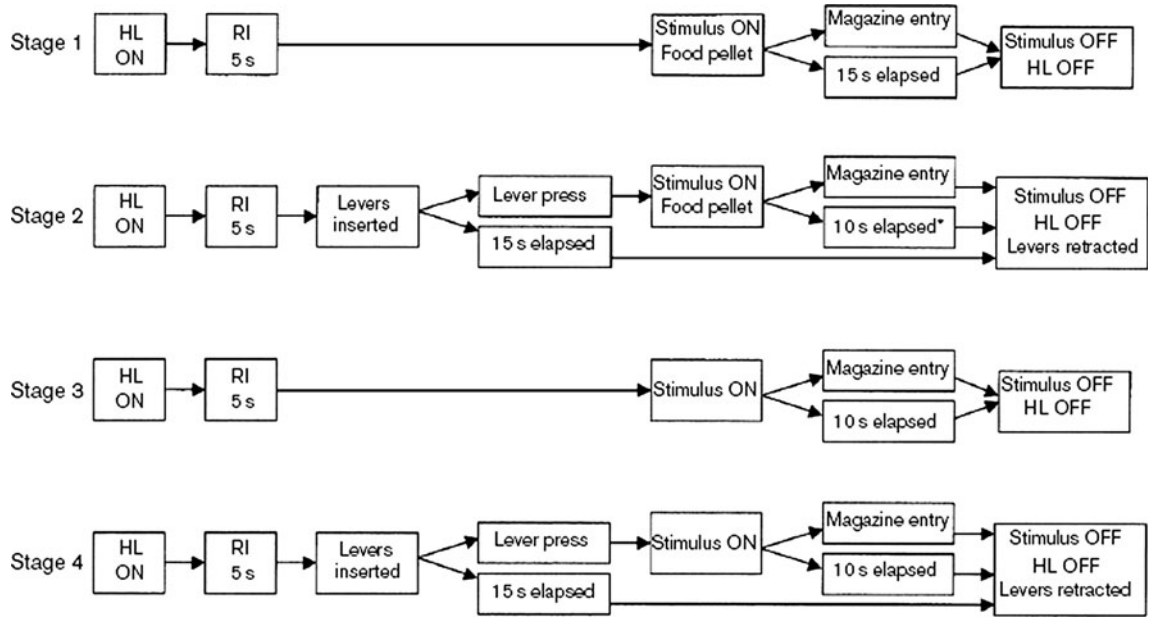

Fig. 1 A schematic diagram of the organization of a trial in each of the different training stages of the post-training signal attenuation procedure. *HL* house light, *RI* random interval. *Asterisk* On the first day of lever-press training (day 5), this time limit was 15 s

each day, each rat was trained until it attained 30 collected trials (that is, trials on which the rat inserted its head into the food magazine during stimulus presentation) or until a total of 40 trials were reached. The number of collected trials and the total number of trials were recorded.

Stage 2 Lever-press training. On day 4, rats received a session of pre-training using a free-operant schedule. Throughout this session, the house lights were on and one lever was present in the operant box. Responding on this lever (reinforced lever, RL) resulted in the delivery of a single food pellet into the magazine, accompanied by the presentation of the compound stimulus (magazine light and tone). The stimulus was turned off after the rat's head entered the food magazine or after 15 s from the rat's first lever press had elapsed. The lever designated as RL was counterbalanced over subjects and remained the same for each rat over the entire experimental procedure. Each rat was trained until it completed 30 trials, that is, pressed the lever and inserted its head into the food magazine during stimulus presentation. Rats that failed to attain 30 completed trials within 30 min were returned to the test chamber at the end of the day for an additional session. On days 5–7, rats were trained to lever press in a discrete-trial procedure (Fig. 1). Each rat was trained until it completed 40 trials, that is, pressed the lever and inserted its head into the food magazine during stimulus presentation or for a total of 60 trials. In order to assess acquisition of the lever-press response, the number of trials on which the rat did not press the RL (unpressed trials) and the number of trials on which the rat pressed the RL without inserting its head into the food magazine (uncompleted trials) were recorded in addition to the number of completed trials. In order to assess rats' tendency for excessive lever pressing, the number of lever presses on the NRL and the number of lever presses on the RL after the first response (extra lever presses, ELP) were recorded. The latter measure was further subdivided into ELP in uncompleted trials (that is, ELP not followed by insertion of the head into the food magazine, ELP-U) and ELP in completed trials (ELP-C). In addition, the number of nose pokes was recorded. Rats were randomly allocated to the different drug groups at the end of this stage. Analysis of the number of ELP-C was carried to make sure that there are no significant differences between the groups on this behavioral measure.

Stage 3 Signal attenuation. On days 8–10, with the levers retracted, rats were exposed to the presentation of the compound stimulus as on days 1–3, but no food was delivered to the food magazine (Fig. 1). Rats received 30 such trials on each day, and the number of collected trials

was recorded. Rats that had more than 13 collected trials on the last day of signal attenuation were returned to the test chamber at the end of the day for an additional session.

Stage 4 Test. On day 11, rats were trained as in the lever-press training stage, except that no food was delivered to the food magazine, that is, pressing the lever resulted in the presentation of the compound stimulus only (Fig. 1). The session lasted for 50 trials. The behavioral measures recorded were the same as in the lever-press training stage. Compulsive lever pressing is operationally defined as the number of ELP-U in the test stage of the post-training signal attenuation procedure.

Regular extinction Rats were run exactly as in the post-training signal attenuation procedure, with the exception that they did not undergo the signal attenuation stage. On these days, rats were brought to the laboratory and left in their home cages for a period equivalent to the average duration of the signal attenuation stage.

Drugs

Physostigmine (Sigma, Rehovot, Israel) was dissolved in saline to doses of 0.05, 0.10, and 0.15 mg/kg and administered subcutaneously (s.c.) in a volume of 1 ml/kg 30 min before the beginning of the test stage. Nicotine (Sigma, Rehovot, Israel) was dissolved in saline to doses of 0.03, 0.06, 0.01, 0.30, 0.60 and 1.00 mg/kg and administered s.c. in a volume of 1 ml/kg 20 min before the beginning of the test stage. Mecamylamine (Sigma, Rehovot, Israel) was dissolved in saline to doses of 1, 3, 5, and 8 mg/kg and administered s.c. in a volume of 1 ml/kg 30 min before the beginning of the test stage. Oxotremorine (Sigma, Rehovot, Israel) was dissolved in saline to doses of 0.0075, 0.0150, and 0.0300 mg/kg and administered i.p. in a volume of 1 ml/kg 30 min before the beginning of the test stage. Scopolamine (Sigma, Rehovot, Israel) was dissolved in saline to doses of 0.15, 0.50, 1.00, and 1.50 mg/kg and administered s.c. in a volume of 1 ml/kg 30 min before the beginning of the test stage. No-drug control rats were administered with the corresponding vehicle. Drug doses were selected on the basis of previous studies which showed that similar doses were effective in altering operant behavior including lever-press responding, without completely abolishing it (Barak and Weiner 2007; Carnicella et al. 2005; Jones et al. 1995; Maehara et al. 2008; Mirza and Stolerman 1998, 2000; Tizabi et al. 2002).

Statistical analysis

Rats' performance on the test stage was analyzed using analysis of variance (ANOVA) with a main factor of dose (experiments 1, 3, 5, 7, and 9) or of dose and procedure (experiments 2, 4, 6, 8, and 10) performed on the number of ELP-C and ELP-U as well as on the number of completed, uncompleted, and unpressed trials and the number of nose-pokes and presses on the non-reinforced lever. Significant dose effects (or dose × procedure interactions) were followed by post hoc least significant difference comparisons comparing each of the drug-treated groups with the vehicle group. For all comparisons, significance was assumed at $p<0.05$.

Although drugs were administered only prior to the test stage, rats' performance on the lever-press training and signal attenuation stages was also analyzed to ensure that differences in performance at the test stage were not a result of an earlier difference. For the former, the number of ELP-C and unpressed trials on the last day of lever-press training were analyzed (as all rats had 40 completed trials and almost no uncompleted trials, the variability of all other variables was too low to enable statistical analysis). Performance on the signal attenuation stage was analyzed using a mixed ANOVA

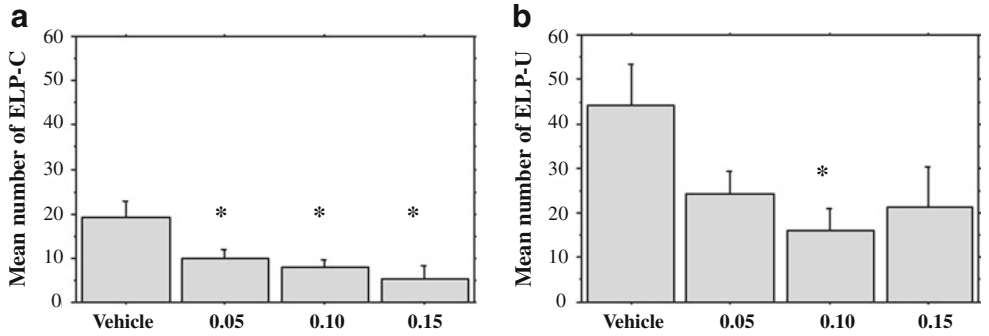

Fig. 2 Mean and standard error of the number of extra lever presses that **a** were followed by magazine entry (extra lever presses in completed trials, ELP-C), and **b** were not followed by magazine entry (extra lever presses in uncompleted trials, ELP-U) of rats treated with vehicle or 0.05, 0.10, or 0.15 mg/kg physostigmine on the test day of the PTSA procedure. *$p<0.05$, significantly different from the vehicle group

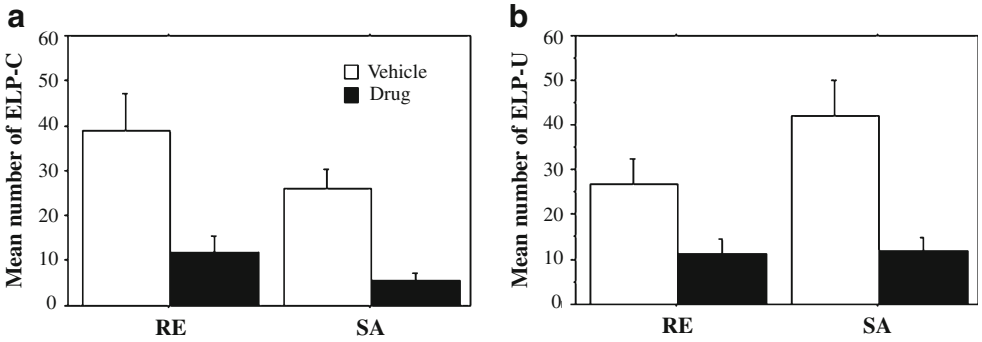

Fig. 3 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 0.1 mg/kg of physostigmine on the test day of the PTSA and regular extinction (RE) procedures

performed on the number of completed trials on the three sessions of the signal attenuation stage.

Results

Table 1 presents the number of rats allocated to each experiment, the number of rats that were excluded from each experiment, the doses used, and the final number of rats in each group.

Experiment 1

The effects of acute administration of 0.05, 0.10, and 0.15 mg/kg of the acetylcholinesterase inhibitor physostigmine in the PTSA procedure.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.7). Figure 2 presents the mean number of extra lever presses that were followed by magazine entry (ELP-C, Fig. 2a) and those that were not followed by magazine entry (ELP-U, Fig. 2b) in physostigmine- and vehicle-treated rats undergoing the test stage of the PTSA procedure. Physostigmine decreased the number of ELP-C [$F(3,32)=3.7$, $p<0.05$] and tended to decrease the number of ELP-U [$F(3,32)=2.71$, $p=0.06$, see Fig. 2 for the results of the post hoc analyses]. Because at a dose of 0.10 mg/kg physostigmine exerted the strongest effect on ELP-U, this dose was selected for further testing in the regular extinction procedure.

Experiment 2

The effects of acute administration of 0.1 mg/kg of the acetylcholinesterase inhibitor physostigmine in the PTSA and regular extinction procedures.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.7). Figure 3 presents the mean number of ELP-C (Fig. 3a) and ELP-U (Fig. 3b) in physostigmine- and vehicle-treated rats undergoing the test stage of the PTSA procedure or the regular extinction procedure. Physostigmine decreased the number of ELP-C and the number of ELP-U in the two procedures [ELP-C: dose: $F(1,42)=22.54$, $p<0.0001$; procedure: $F(1,42)=3.64$, $p=0.06$; procedure × dose interaction: $F(1,42)=0.39$, $p=0.53$; ELP-U: dose: $F(1,42)=18.46$, $p<0.05$; procedure: $F(1,42)=2.28$, $p=0.13$; procedure × dose interaction: $F(1,42)=1.84$, $p=0.18$].

Experiment 3

The effects of 0.03, 0.06, 0.10, 0.30, 0.60, and 1.00 mg/kg of the nicotinic agonist nicotine in the PTSA procedure.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.86). Figure 4 presents the mean number of ELP-C (Fig. 4a) and ELP-U (Fig. 4b) in nicotine- and vehicle-treated rats undergoing the test stage of the PTSA procedure. Nicotine did not affect the number of ELP-C [$F(6,106)=1.33$, $p=0.25$] but

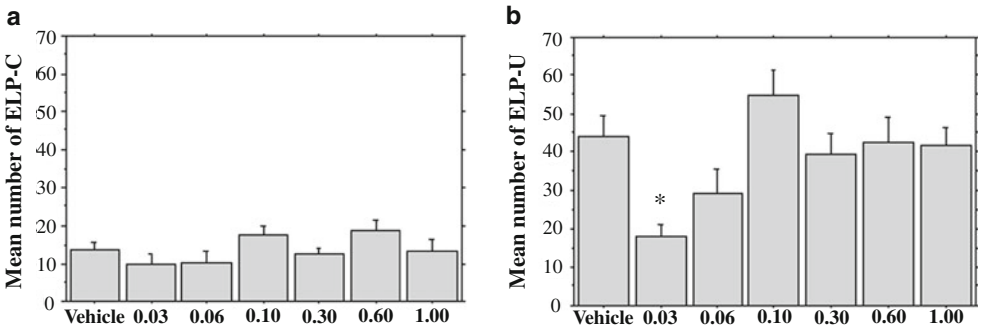

Fig. 4 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 0.03, 0.06, 0.10, 0.30, 0.60, or 1.00 mg/kg of nicotine on the test day of the PTSA procedure. *$p<0.05$, significantly different from the vehicle group

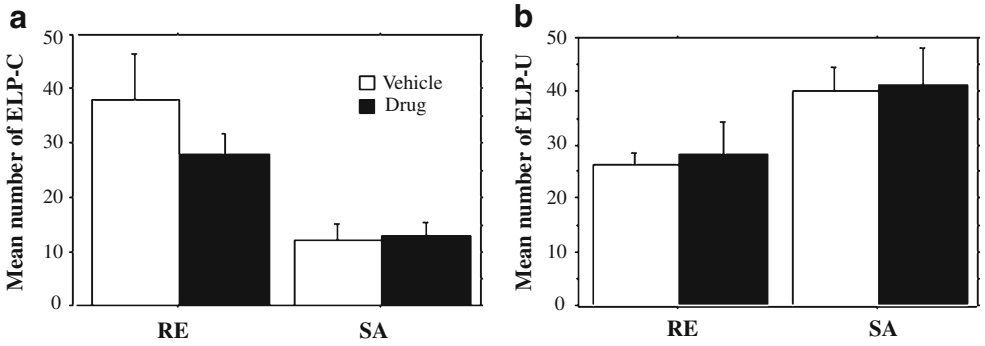

Fig. 5 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 0.03 mg/kg of nicotine on the test day of the PTSA and RE procedures

tended to decrease the number of ELP-U [$F(6,106)=2.03$, $p=0.07$] especially at low doses (see Fig. 4 for the results of the post hoc comparisons). Because at a dose of 0.03 mg/kg nicotine exerted the strongest effect on ELP-U, this dose was selected for further testing in the regular extinction procedure.

Experiment 4

The effects of 0.03 mg/kg nicotine in the PTSA and regular extinction procedures.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.92). Figure 5 presents the mean number of ELP-C (Fig. 5a) and ELP-U (Fig. 5b) in nicotine- and vehicle-treated rats undergoing the test stage of the PTSA procedure or the regular extinction procedure. Nicotine did not affect the two types of lever press in the two procedures [ELP-C: dose: $F(1,44)=0.85$, $p=0.36$; procedure: $F(1,44)=16.32$, $p<0.05$; procedure × dose interaction: $F(1,44)=1.14$, $p=0.29$; ELP-U: dose: $F(1,44)=0.07$, $p=0.78$; procedure: $F(1,44)=6.6$, $p<0.05$; procedure × dose interaction: $F(1,44)=0.005$, $p=0.94$].

Experiment 5

The effects of acute administration of 1, 3, and 5 mg/kg of the nicotinic antagonist mecamylamine in the PTSA procedure.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.8). Figure 6 presents the mean number of ELP-C (Fig. 6a) and ELP-U (Fig. 6b) in mecamylamine- and vehicle-treated rats undergoing the test stage of the PTSA procedure. Mecamylamine had no effect on the two types of lever presses [ELP-C: $F(3,41)=0.75$, $p=0.52$; ELP-U: $F(3,41)=0.7$, $p=0.55$].

Because this drug had no effect on rats' behavior, we tested the effects of a higher dose. Because in a pilot study we found that at doses of 10 and 12.5 mg/kg mecamylamine almost completely abolished lever-press responding, experiment 6 tested the effects of 8 mg/kg mecamylamine in the PTSA and regular extinction procedures.

Experiment 6

The effects of 8 mg/kg mecamylamine in the PTSA and regular extinction procedures.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.92). Figure 7 presents the mean number of ELP-C (Fig. 7a) and ELP-U (Fig. 7b) in mecamylamine- and vehicle-treated rats undergoing the test stage of the PTSA procedure or the regular extinction procedure. Mecamylamine did not affect the two types of lever press in the two procedures [ELP-C: dose: $F(1,37)=0.72$, $p=0.4$; procedure: $F(1,37)=29.67$, $p<0.0001$; procedure × dose interaction: $F(1,37)=0.17$, $p=0.67$; ELP-U: dose: $F(1,37)=$

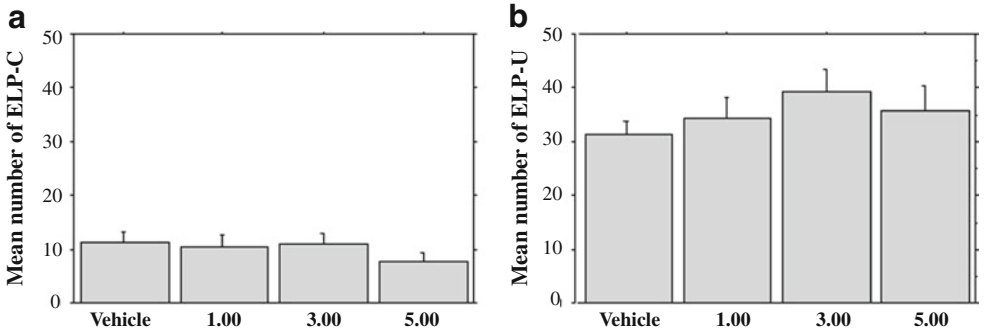

Fig. 6 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 1, 3, or 5 mg/kg of mecamylamine on the test day of the PTSA procedure

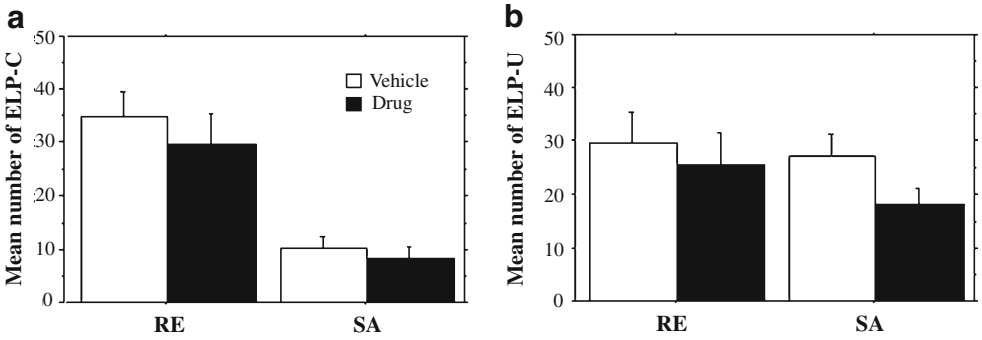

Fig. 7 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 8 mg/kg of mecamylamine on the test day of the PTSA and RE procedures

1.67, p=0.2; procedure: F(1,37)=0.84, p=0.36; procedure × dose interaction: F(1,37)=0.27, p=0.6].

Experiment 7

The effects of acute administration of 0.0075, 0.0150, and 0.0300 mg/kg of the muscarinic agonist oxotremorine in the PTSA procedure.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.7). Figure 8 presents the mean number of ELP-C (Fig. 8a) and ELP-U (Fig. 8b) in oxotremorine- and vehicle-treated rats undergoing the test stage of the PTSA procedure. Oxotremorine decreased the number of ELP-C [F(3,24)=2.92, p=0.05] and of ELP-U [F(3,24)=2.94, p=0.05, see Fig. 8 for the results of the post hoc comparisons]. Because at a dose of 0.0300 mg/kg oxotremorine exerted the strongest effect on ELP-U, this dose was selected for further testing in the regular extinction procedure.

Experiment 8

The effects of 0.03 mg/kg of the muscarinic agonist oxotremorine in the PTSA and regular extinction procedures.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.8). Figure 9 presents the mean number of ELP-C (Fig. 9a) and ELP-U (Fig. 9b) in oxotremorine- and vehicle-treated rats undergoing the test stage of the PTSA or regular extinction procedures. Oxotremorine decreased the number of ELP-C in the two procedures but did not have a significant effect on the number of ELP-U [ELP-C: dose: F(1,26)=6.51, p<0.05; procedure: F(1,26)=21.66, p<0.0001; procedure × dose interaction: F(1,26)=0.59, p=0.44; ELP-U: dose: F(1,26)=3.69, p=0.07; procedure: F(1,26)=0.41, p=0.52; procedure × dose interaction: F(1,26)=1.87, p=0.18].

Experiment 9

The effects of 0.15, 0.50, and 1.50 mg/kg of the muscarinic antagonist scopolamine in the PTSA procedure.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.85). Figure 10 presents the mean number of ELP-C (Fig. 10a) and ELP-U (Fig. 10b) in scopolamine- and vehicle-treated rats undergoing the test stage of the PTSA procedure. Scopolamine decreased the number of ELP-C [F(3,40)=9.5, p<0.0001] and of ELP-U [F(3,40)=5.56, p<0.05, see Fig. 10 for the results of the post hoc comparisons]. As the effects of scopolamine on ELP-U were strongest at 0.5 and 1.5 mg/kg, but at 1.50 mg/kg scopolamine also exerted a strong effect on the number of ELP-C, experiment 10 tested the effects of 0.50 and 1.00 mg/kg of scopolamine in the PTSA and regular extinction procedures.

Experiment 10

The effects of 0.5 and 1.0 mg/kg of the muscarinic antagonist scopolamine in the PTSA and regular extinction procedures.

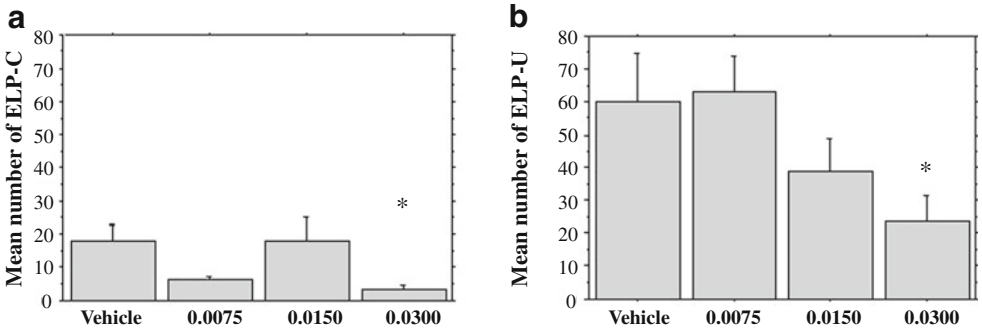

Fig. 8 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 0.0075, 0.0150, or 0.0300 mg/kg of oxotremorine on the test day of the PTSA procedure. *p<0.05, significantly different from the vehicle group

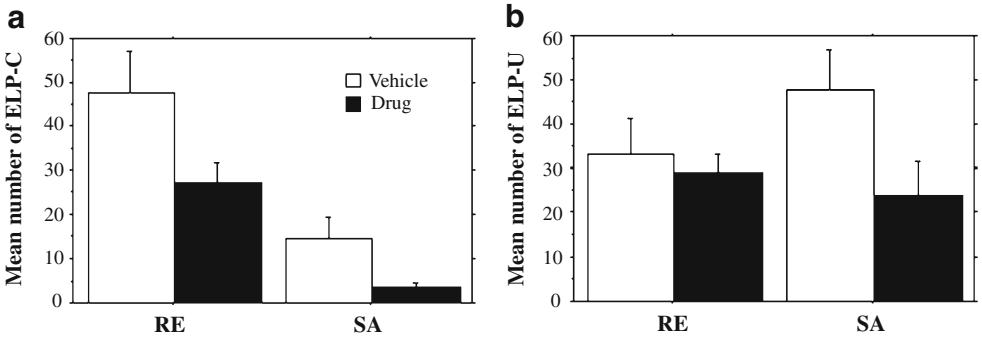

Fig. 9 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 0.03 mg/kg of oxotremorine on the test day of the PTSA and RE procedures

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown, all p's>0.89). Figure 11 presents the mean number of ELP-C (Fig. 11a) and ELP-U (Fig. 11b) in scopolamine- and vehicle-treated rats undergoing the test stage of the PTSA or regular extinction procedure. Scopolamine decreased the number of ELP-C in the two procedures (although the effect in the PTSA procedure was obtained at a lower dose) [dose: $F(2,52)=3.61$, $p<0.05$; procedure: $F(1,52)=3.89$, $p=0.05$; procedure × dose interaction: $F(2,52)=1.41$, $p=0.25$] and, in addition, decreased the number of ELP-U in both the PTSA and regular extinction procedures [dose: $F(2,52)=15.07$, $p<0.0001$; procedure: $F(1,52)=3.08$, $p=0.085$; procedure × dose interaction: $F(2,52)=2.1$, $p=0.13$].

Discussion

The present study tested whether manipulations of the cholinergic system by acute systemic administration of cholinergic agonists and antagonists affect compulsive lever pressing. Our main finding is that none of the drugs tested exerted a selective anti- or pro-compulsive effect in the signal attenuation model (see Table 2 for a summary of the results). Rather, at the doses tested, the different drugs either had no effect on lever press behavior (i.e., nicotine and mecamylamine) or decreased it in a nonselective manner (i.e., physostigmine, oxotremorine, and scopolamine). The latter effect may either reflect a general decrease in lever-press responding or facilitated extinction of the lever-press response. Although cholinergic manipulations have been reported to affect both lever-press responding and extinction, the pattern of reported effects only partly overlaps with the findings of the present study. Specifically, acute administration of physostigmine and oxotremorine was found to decrease lever-press responding (Mirza and Stolerman 2000), in line with the present findings. Administration of acetylcholinesterase inhibitors facilitated extinction (Banks and Russell 1967; Glazer 1972) in line with the present finding, although these studies used chronic administration while the present study used acute administration. Acute and chronic administration of nicotine (at a dose of 0.2 mg/kg) was found to facilitate extinction of active avoidance and of conditioned fear (Driscoll and Battig 1970; Tian et al. 2008), and chronic nicotine administration was reported to increase lever pressing (Raiff and Dallery 2009), in contrast to nicotine's lack of effect in the present study (at doses between 0.03 and 1.00 mg/kg). Similarly, whereas previous studies reported retarded extinction of lever-press responding following acute administration of scopolamine (Carlton 1961, 1963; Hearst 1959; McCoy 1972; Plotnik et al. 1976), we have found facilitated extinction. A possible reason for the inconsistent findings may be differences in the reinforcer used, as at least the effects of scopolamine were found to depend on the type of reinforcer (retarded extinction was evident with water or milk reward but not with a food reward; Morley and Russin 1978; Olds 1970).

Our finding that nicotine does not affect "compulsive" responding in a rat model of OCD contradicts a previous report that nicotine has an anti-compulsive effect in the quinpirole rat model of OCD (Tizabi et al. 2002). This discrepancy may be a result of the different administration regimen of the drug (once at the present study and twice at Tizabi

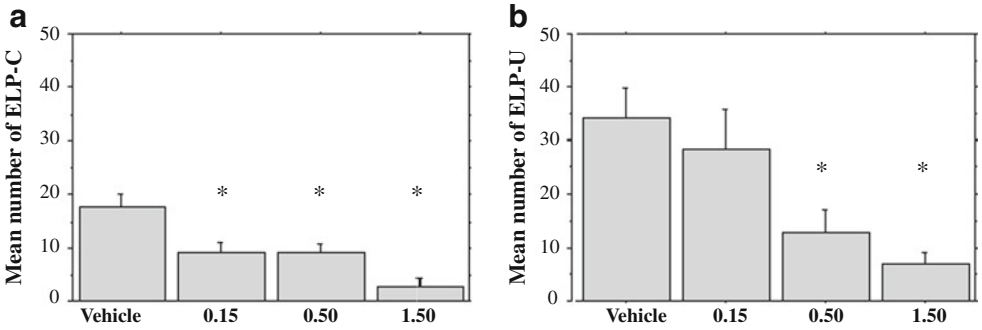

Fig. 10 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 0.15, 0.50, or 1.50 mg/kg of scopolamine on the test day of the PTSA procedure. *$p<0.05$, significantly different from the vehicle group

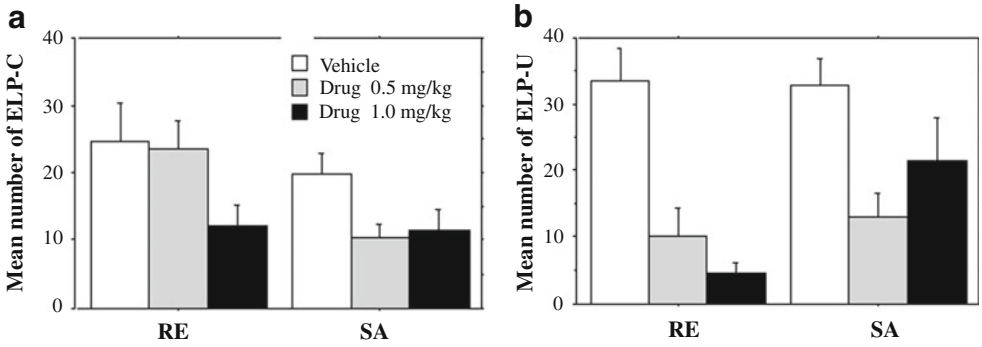

Fig. 11 Mean and standard error of the number of **a** ELP-C and **b** ELP-U of rats treated with vehicle or 0.5 or 1.0 mg/kg of scopolamine on the test day of the PTSA and RE procedures

et al.'s study). Alternatively, this discrepancy may be related to the differential sensitivity of the quinpirole and signal attenuation models. Specifically, on the basis of the finding that compulsive-like behavior in the quinpirole model is only weakly and temporarily affected by the serotonin reuptake inhibitor (SRI) clomipramine, whereas compulsive-like behavior in the signal attenuation model is reduced by several SSRIs, it has been suggested that the quinpirole model may be used to detect drugs with beneficial effects for patients who are nonresponsive to S/SRIs, whereas the signal attenuation model may be more relevant to SSRI responders (Albelda and Joel 2012). If this is true, the pattern of effects of nicotine in the two models may suggest that this drug may be beneficial to SSRI nonresponders but not to SSRI responders.

There are no other studies that tested the effects of cholinergic drugs in animal models of OCD, but there are several studies that reported drug effects on perseverative behaviors, which have been suggested to model compulsive behaviors (Boulougouris et al. 2008; Chudasama et al. 2003; Clarke et al. 2007). Specifically, perseveration in a probability discounting task was increased by acute administration of nicotine (Mendez et al. 2012). Perseveration in the five-choice serial reaction time task was not affected by acute administration of scopolamine, physostigmine, oxotremorine, and several nicotinic antagonists (Grottick and Higgins 2000; Mirza and Stolerman 2000), while acute mecamylamine administration was reported to have no effect (Mirza and Stolerman 2000) or to increase perseveration in this task (Grottick and Higgins 2000). In contrast to the lack of effect of scopolamine in the five-choice serial reaction time task, acute administration of this drug was found to increase perseveration in reversal (Wongwitdecha and Marsden 1996). To this complex pattern of effects, we can add the present observation that cholinergic manipulations did not retard rats' ability to respond to the change of contingencies in the regular extinction procedure. Taken together, these results clearly demonstrate that perseverative behavior in different tasks may be subserved by different neural mechanisms, only some of which are affected by cholinergic manipulations. However, as different drugs were tested in different procedures, it is difficult to draw a coherent picture or reach conclusions regarding the cognitive mechanisms that are modulated by cholinergic manipulations and those that are not.

In summary, as detailed in the "Introduction," the signal attenuation model may serve to screen drugs for anticompulsive activity using acute administration regimen because under these conditions only drugs with a known anticompulsive effect have been shown to induce an anticompulsive effect in the model (Albelda and Joel 2012). The present findings that several cholinergic manipulations do not exert an anti- or pro-compulsivity effect in the signal attenuation rat model of OCD suggest that cholinergic manipulations of the types used in the present study may not be effective in the treatment of OCD patients. Yet, as detailed above, this conclusion may apply only to SSRI responsive patients. In addition, the present findings demonstrate the pharmacological selectivity of the signal attenuation model. Whether this contributes to the model's validity or not will become clear only when more data are available on the role of the cholinergic system in OCD.

Table 2 Summary of results

Drug	ELP-U		ELP-C	
	PTSA	RE	PTSA	RE
Physostigmine (acetylcholinesterase inhibitor)	↓	↓	↓	↓
Nicotine (nicotinic agonist)	–	–	–	–
Mecamylamine (nicotinic antagonist)	–	–	–	–
Oxotremorine (muscarinic agonist)	↓	↓	↓	↓
Scopolamine (muscarinic antagonist)	↓	↓	↓	–

PTSA post-training signal attenuation, *RE* regular extinction

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