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Strain-dependent effects of cognitive enhancers in the mouse

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Summary. A series of cognitive enhancers (CEs) have been reported to increase spatial memory in rodents, information on behavioral effects, however, is limited. The aim of the study was therefore to examine the behavioral effects of three CEs in two well-documented inbred mouse strains.

C57BL/6J and DBA/2 mice were administered intraperitonial. D-cycloserine (DCS; NMDA receptor agonist), 1-(4-Amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride (RS67333; 5HT4-receptor agonist), and (R)-4-{[2-(1-methyl-2-pyrrolidinyl)ethyl]thio}phenol hydrochloride (SIB-1553A; β-4-nicotinic receptor agonist) and tested in the open field (OF), elevated plus maze (EPM), neurological observational battery and rota-rod. Cognitive performance was tested in the Morris water maze.

All compounds modified behavioral performance in the OF, DCS showed an anxiolytic effect in the EPM, and differences in the observational battery were observed i.e. vestibular drop was decreased by SIB-1553A and RS67333 treatment in C57BL/6J and increased with DCS treatment in DBA/2 mice. In the rota rod SIB-1553A improved motor performance.

DCS effects on learning and memory was comparable to controls whereas the other compounds impaired performance in the Morris water maze.

In conclusion, behavioral testing of CEs in the mouse revealed significant changes that may have to be taken into account for evaluation of CEs, interpretation of cognitive studies and warrant further neurotoxicological studies. Moreover, strain-dependent differences were observed that in turn may confound results obtained from behavioral and cognitive testing.

Keywords: Inbred strains – D-cycloserine – RS67333 – SIB-1553A – Behavioral testing – Morris water maze

Abbreviations: CEs, cognitive enhancers; DCS, D-cycloserine; EPM, Elevated plus maze; 5-HT, 5-Hydroxytryptamine; MWM, morris water maze; NMDA, N-methyl-D-aspartate; OF, open field; RS67333, 1-(4-Amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride; SIB-1553A, (R)-4-{[2-(1-methyl-2-pyrrolidinyl)ethyl]thio} phenol hydrochloride

1. Introduction

A cognitive enhancer (CE) is any compound that will increase the final level of performance (Wenk and Olton, 1989). The development of such substances has clinical relevance for augmenting cognitive performance or treatment of cognitive dysfunction associated with aging, neurodegenerative diseases and psychiatric disorders. A series of compounds have been reported as potential memory or CEs and some of them have been already evaluated in clinical trials (Laake and Oeksengaard, 2002; Froestl et al., 2004; Tuominen et al., 2006). Modulation of several receptor systems has been studied:

1.1 Agonist N-methyl-D-aspartate (NMDA) receptors

N-methyl-D-aspartate (NMDA) receptors are involved in pathways for learning and memory formation and several pharmacological studies, which have shown that antagonists of the NMDA receptor complex administered before the training session disrupted certain types of learning and memory (Morris et al., 1986; Tang and Ho, 1988; Malenfant et al., 1991) support this conclusion.

D-cycloserine (DCS) was originally used as an antibiotic agent for treatment of tuberculosis and later characterized as a partial agonist at the NMDA receptor glycine-binding site (Hood et al., 1989; Henderson et al., 1990; Hood et al., 1990; Emmett et al., 1999). DCS appears to be a potent CE at doses lower than those required for antibacterial activity (Monahan et al., 1989; Baxter et al., 1994; Land and Riccio, 1999). It was reported as a performance enhancer in different tasks assessing learning and memory (Thompson et al., 1992; Quartermain et al., 1994; Lelong et al., 2001). At doses used in most of the studies, DCS has no apparent effect upon the motivational state or

the motor skills of experimental animals (Herberg et al., 1990; Baxter et al., 1994; McBain et al., 1994).

In behavioural studies, high doses of DCS (200–300 mg/kg) lead to anxiolytic-like activity in Sprague-Dawley rats (Anthony et al., 1993; Klodzinska et al., 2000). Low doses (10–30 mg/kg) showed an anxiogenic effect by preventing ethanol-induced anxiolytic effects in the plus-maze test at doses 3–12 mg/kg (Moraes Ferreira et al., 1997) and by changing behavior in low anxiety male Wistar rats in the elevated plus maze (EPM) (Ho et al., 2005).

Most investigators have observed that DCS administration in rats and mice promoted spatial learning (Monahan et al., 1989; Baxter et al., 1994). Retention of a thirst-motivated linear maze task was enhanced by the administration of 10, 20, or 80 mg/kg of DCS to mice, immediately following training. In addition, a dose of 3 mg/kg of DCS administered 20 min before the training facilitated acquisition in this task. Acute post-training injections failed to facilitate retention if mice were pre-treated with DCS (3 mg/kg) for 15 days prior to training in the maze. The study not only indicated that DCS administration enhances consolidation and retrieval of memory but also that desensitization may occur with chronic exposure to the drug (Quartermain et al., 1994). Trained young mice injected with 20 mg/kg of DCS also exhibited increased memory retention as revealed by an electric shock-motivated T-maze task (Flood et al., 1992).

Different results were obtained by training rats in the Morris water maze (MWM), used to assess spatial memory. Pitkänen et al. (1995) treated rats with DCS and assessed them in the Morris water maze (MWM) task that did not improve spatial memory. On the other hand, data obtained by Lelong (2001) revealed that training performance of treated rats in MWM improved significantly.

1.2 Agonist β-4-nicotinic receptors

Nicotinic receptors are ligand-gated ion channels (Changeux et al., 1998; Changeux, 1990a) that include a variety of receptor subtypes, important for a variety of neurobehavioral functions including cognitive function (Changeux, 1990b). Indeed, nicotinic receptors are thought to play a direct or indirect role in the pathophysiology of different diseases such as schizophrenia, attention deficit hyperactivity disorder, Parkinson's disease and Alzheimer's disease.

(R)-4-{[2-(1-Methyl-2-pyrrolidinyl)ethyl]thio}phenol hydrochloride (SIB-1553A) is a neuronal Acetylcholine receptor ligand with agonist selectivity for β4-subunit containing human neuronal Acetylcholine receptors (Decker et al., 1994). SIB-1553A enhances cognitive performance in rodents with cholinergic dysfunction coupled with an improved safety profile relative to nicotine. Moreover, SIB-1553A has been shown to stimulate the release of acetylcholine, dopamine and norepinephrine from the hippocampus and frontal cortex in young adult rats (Rao et al., 2003a, b).

SIB-1553A treatment improved performances in spatial and non-spatial working memory tasks (Bontempi et al., 2001, 2003).

1.3 5-HT$_4$ receptor agonist

5-Hydroxytryptamine (5-HT) receptors are G-protein coupled seven transmembrane receptors that activate an intracellular second messenger cascade (cAMP), which plays an important role in hippocampal long term potentiation by activating protein kinase A (PKA). These receptors are expressed in limbic areas.

The effects of 5-HT$_4$ receptor ligands have been studied both, in normal and cognitively impaired animals, and it has been demonstrated that a functional interaction between the serotonergic system mediated via 5-HT$_4$ receptors and the cholinergic system associated with cognitive processes exists. It is suggested that their possible role in learning and memory may be through modulation of acetylcholine release in these structures (Consolo et al., 1994).

Moreover, postmortem studies revealed that the hippocampal 5-HT$_4$ receptor density declines in patients with cholinergic and memory alterations such as those observed in Alzheimer's disease (Wong et al., 1996).

1-(4-Amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride (RS67333), a highly selective 5-HT$_4$ receptor agonist, improved spatial memory in primates and rats (Fontana et al., 1997; Terry et al., 1998; Lelong et al., 2001; Orsetti et al., 2003). Pretraining treatment of rats with 5-HT$_4$ receptor agonists improved social learning and shortened the delay to obtain maximal performances in an olfactory associative test (Marchetti et al., 2000).

The anxiolytic-like effects of a variety of 5-HT receptor agonists and antagonists have been intensively studied in animal models. However, no direct effects of 5-HT$_4$ receptor agonist have been reported.

However important development of CEs may be, no systematic study on side effects, adverse effects or neuro-

toxicological evaluation was carried out so far. It was the aim of the present study to evaluate probable adverse effects of CEs on behavioral functions including anxiety-related, motor and exploratory behaviour, cognitive function, basic neurological and psychomotor status and motor performance in two widely-used mouse inbred strains.

2. Materials and methods

2.1 Animals

C57BL/6J and DBA/2 mice, male, aged 10–14 weeks were used. Mice were bred and maintained in cages made of Makrolon and filled with autoclaved woodchips in the Core unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna. An autoclaved standard rodent diet (Altromin 1314ff) and water acidified to pH 3 from automatic valves or in bottles were available ad libitum. Room temperature was $22 + 1\,^{\circ}C$ and relative humidity was $50 \pm 10\%$. Ventilation with 100% fresh air resulted in an air change rate of 15 times per hour. The room was illuminated with artificial light at an intensity of about 200 l× in 2 m from 5 am to 7 pm. Behavioral tests were performed between 8 am and 1 pm.

2.2 Compounds

Animals were treated intra-peritoneal before daily testing. Mice were divided into three treatment and one control (vehicle) group 0.9% NaCl solution. The doses and time of injection was chosen through literature search (see Table 1).

DCS was purchased from Sigma-Aldrich (Austria). RS67333 was purchased from Tocris Bioscience; SIB-1553A was supplied by Chemical Synthesis and Drug Supply Program of the National Institute of Mental Health (NIMH). Drugs were daily fresh prepared.

2.3 Behavioral training procedures

2.3.1 Behavioral observational screen

2.3.1.1 Basic neurological and physiological observational assessment: The procedure followed the set up by Irwin (1968). A battery of tests was applied to reveal defects in gait or posture, changes in muscle tone, grip strength, visual acuity and temperature. To complete the assessment, vitally important reflexes were scored. In addition, during the manipulations, incidences of abnormal behaviour, fear, irritability, aggression, excitability, salivation, lacrimation, urination and defecation were recorded.

Table 1. Drugs used in the experiments and the corresponding injection times and doses, taken through literature search

Drugs	Time of injection	Doses	References
DCS	30 min before trials	10 mg/kg	Lelong et al. (2001)
RS67333	30 min before trials	1 mg/kg	Lelong et al. (2001)
SIB-1553A	20 min before trials	2, 5 mg/kg	Bontempi et al. (2001, 2003)

2.3.1.2 Rota rod: The rota rod (Rota Rod "Economex", Columbus Instruments, Ohio, USA) tests balance and coordination and comprises a rotating drum which is accelerated from 4 to 40 rpm over the course of 5 min. The time at which each animal fell from the drum was recorded. Each animal received three pre-training trials. Subsequently, each mouse completed three more consecutive trials and the longest time on the drum was used for analysis (Rogers et al., 1999).

2.3.1.3 Elevated plus-maze (EPM): A paradigm was used for the study of anxiety in mice. Animals were observed for 5 min on a maze consisting of 4 arms (each 30 cm long and 5 cm wide) fixed in a height of 54 cm. The arms are interconnected by a 5×5 cm wide central area. Two arms contain 15 cm high side and end walls (= closed arm). Standard parameters reflecting anxiety related behaviour (i.e. closed arm entries, time spent in closed arms, frequency of defecation) were evaluated (Weitzdoerfer et al., 2004).

2.3.1.4 Open field (OF): Mice were observed using a video monitoring system consisting of a video camcorder (1/3″ SSAM HR EX VIEW HAD) coupled to computational tracking system TiBeSplit in an arena (40×40 cm long; with 70 cm high walls) for 10 min. The individual mice were placed in the middle of the chamber for each trial. Standard parameters for locomotor activity (i.e. total distance covered, average speed, amount of large movement, amount of local movement, resting time, frequency of spontaneous changes of direction) and exploratory behaviour (i.e. rearing, crossing the centre, time spent in the centre) were recorded (Weitzdoerfer et al., 2004).

2.3.2 Morris water maze learning task

MWM consisted of a circular pool (122 cm diameter, walls 76 cm depth) in which mice were trained to escape from water by swimming to a hidden platform (1.5 cm beneath water surface) whose location could be only identified using distal extra-maze cues attached to the room walls. Water temperature was maintained at $21 \pm 1\,^{\circ}C$.

The pool was divided into four quadrants by a computerized tracking/image analyzer system (video camcorder: 1/3″ SSAM HR EX VIEW HAD coupled to computational tracking system: TiBeSplit). The platform was placed in the middle of the SW quadrant and remained at the same position during the whole experiment.

The spatial learning task consisted of 4 training trials per day and 4 training days. Mice were released with their heads facing the pool wall from the four compass locations (NE, NW, SW, and SE; in this order), and allowed to swim and search for the platform for 120 sec. If the mice did not locate the platform after 120 sec, animals were manually placed on the platform and allowed to remain on it for 30 sec. Each animal was then returned to its cage for 10 min before its next trial. On the first training day, mice were given an acclimatization training session in the water maze for 30 sec.

One day after the last training day, subjects received a probe trial, in which the platform was removed. The mouse was released from NE start point and allowed to swim freely for 60 sec. The path that the mouse swam was tracked and analysed for the proportion of swim time and/or path length spent in each quadrant of the pool and swim speed was recorded.

2.4 Experimental design

Firstly, in the open field test, the elevated plus maze assay, the neurological observational battery and rota-rod evaluation were carried out. The order of testing was designed such that the procedures most likely to be affected by prior handling were carried out first (Rogers et al., 1999): EPM, OF, battery of neurological observations and rota rod. Drugs were administered prior to testing. One week later, after completion of the behavioural

observational screen, all mice were evaluated in the MWM to show the effect of CEs on memory formation.

2.5 Statistical analysis

Statistical analysis to reveal between-group differences was performed by unpaired Student's *t*-test. If data violated a principal assumption of a parametric distribution non-parametric Mann–Whitney *U*-test was carried out. Fisher's exact and Chi-square test were used when applicable.

In all instances, a probability level of $P < 0.05$ was considered as statistically significant. All calculations were performed using GraphPad InStat version 3.00 for Windows (GraphPad Software, San Diego CA, USA).

Outliers were excluded from data handling following the principle of Dixon et al. (1953).

3. Results

3.1 Elevated plus maze

As shown in Table 2a, total distance covered in closed arms, time spent in closed arms and resting time was significantly decreased by DCS compared to vehicle control group in inbred C57BL/6J.

3.2 Open field

As shown in Table 2b, total distance covered was significantly increased in DCS in DBA/2 mice. DCS decreased significantly the resting time and the time spent in the margin and increased significantly the number of times crossing the center, the frequency of spontaneous changes of direction and the amount of large movements. In C57BL/6J, DCS increased significantly number of times crossing the center and the amount of rearing and grooming.

DBA/2 mice treated with RS67333 showed no significant differences versus the control group. In C57BL/6J treated mice total distance covered, average velocity, number of times crossing the center, frequency of spontaneous changes of direction and amount of large movements was significantly increased.

3.3 Observational battery

The multitude of results is listed in Table 2c.I and 2c. II.

Vestibular drop, grip strength and body tone was significantly increased in DBA/2 mice treated with DCS. In C57BL/6J mice treated with DCS touch escape, startle response, pelvic elevation and limb tone was significantly increased, while grip strength was decreased. Vestibular drop in C57BL/6J mice treated with SIB-1553A and RS67333 was significantly decreased. Vocalization and limb rotation was significantly increased in DBA/2 mice treated with SIB-1553A. Body tone was increased and urination was decreased in C57BL/6J mice treated with SIB-1553A. Pelvic elevation, Tail elevation and body tone was increased in C57BL/6J mice treated with RS67333. Body

Table 2a. Results of the EPM

C57BL/6J	NaCl ($n = 12$)	DCS ($n = 16$)	SIB1553A ($n = 14$)	RS67333 ($n = 12$)
Closed arm (CA) left	11.3 ± 3.2	11.6 ± 3.8	10.5 ± 2.5	10 ± 3.9
Distance covered in CA (m)	6.1 ± 1.8	$4.9 \pm 1.7^{*}$	6.2 ± 0.9	5.4 ± 1.5
Time spent in CA (s)	199.5 ± 51.8	$153.0 \pm 49.3^{*}$	220.3 ± 25.5	178.7 ± 47.7
Total distance covered (m)	8.1 ± 1.2	8.7 ± 1.6	7.8 ± 1.5	7.8 ± 1.5
Resting time (s)	66.6 ± 5.3	$62.1 \pm 6.4^{*}$	68.0 ± 6.2	66.6 ± 6.2
Amount of large movement (%)	4.5 ± 1.9	4.3 ± 2.7	3.9 ± 2.5	3.7 ± 2.6
Defecation	0.4 ± 0.1	0.4 ± 1.1	0.9 ± 1.3	0.5 ± 1.0
DBA/2	($n = 11$)	($n = 16$)	($n = 11$)	($n = 15$)
Closed arm (CA) left	8.0 ± 5.0	7.6 ± 3.1	6.5 ± 4.3	6.7 ± 1.6
Distance covered in CA (m)	6.5 ± 2.1	6.5 ± 1.4	5.8 ± 1.4	6.0 ± 1.7
Time spent in CA (s)	218.2 ± 54.8	232.6 ± 31.5	219.7 ± 44.5	228.1 ± 43.1
Total distance covered (m)	7.9 ± 1.8	7.4 ± 1.0	7.1 ± 1.2	7.1 ± 1.5
Resting time	71.0 ± 6.8	73.7 ± 3.5	74.9 ± 3.8	72.9 ± 5.5
Amount of large movement (%)	5.7 ± 3.1	5.3 ± 1.8	5.2 ± 1.9	4.4 ± 2.6
Defecation	0.8 ± 1.0	1 ± 1.2	0.4 ± 0.7	1.5 ± 1.7

Asterisks indicate significance of difference of drug-treated mice (C57BL/6J and DBA/2) compared to control group (0.9% NaCl solution), (Student's *t*-test and/or Mann–Whitney *U*-test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P \leq 0.001$)

tone and diarrhea was significantly increased in DBA/2 mice treated with RS67333.

3.4 Rota rod

Performance on the Rota rod was not significantly changed in DBA/2 treated mice compared with the control group. SIB-1553A stayed significantly longer on the drum than its control (see Table 2d).

3.5 Morris water maze task

Figure 1 shows the spatial learning curves presenting mean ± S.D latencies to find the submerged platform for each mouse strain during 4 days of acquisition training and Fig. 2 shows the percentage of time spent in the target quadrant during the probe trial vs. the average of the three control quadrants.

In C57BL/6J inbred strain no significant difference between control and treated groups was found during the first 2 training days. On day 3, DCS completed the trial (within 45.1 ± 35.8) significantly faster than the control group (52.6 ± 18.0). However, no significant performance differences were observed on day 4 (see Fig. 1).

During the consolidation phase (see Fig. 2), C57BL/6J time spent in the target quadrant was measured. SIB-1553A (26.4% ±7.7) and RS67333 (21.3% ±5.0) treated mice showed reduced time spent in the quadrant were the platform used to be (31.8% ±6.4).

DBA/2 mice of the control group failed to learn and showed no preference for the target quadrant on probe trial as shown in Figs. 1 and 2.

On day 3 DBA/2 treated mice (DCS: 53.3 ± 20.7; SIB-1553A: 61.8 ± 23.8) needed significantly more time to reach the platform in comparison with the control group (33.9 ± 26.3). Again, no significant difference of latency to reach the goal was found on day 4.

On the probe trial, DCS (20.5% ±9.1) and SIB-1553A (19.3% ±5.7) treated mice performed worse than the control group (26.0% ±4.3) spending significantly less time in the target quadrant (see Fig. 2). RS67333 showed no

Table 2b. Results of the OF

C57BL/6J	NaCl ($n=12$)	DCS ($n=16$)	SIB1553A ($n=14$)	RS67333 ($n=12$)
Total distance covered (m)	33.6 ± 6.2	34.8 ± 6.5	37.2 ± 11.7	42.4 ± 13.5*
Resting time	46.4 ± 5.6	46:4 ± 6.8	45.3 ± 6.8	42.4 ± 6.9
Average velocity (m/s)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.07 ± 0.02*
No of times crossing the center	7.9 ± 4.4	11.6 ± 4.5*	10.4 ± 4.1	12.8 ± 5.2*
Frequency of sniffing/rearing	93.3 ± 40.3	128.5 ± 38.8*	101.5 ± 41.3	106.3 ± 38.2
Frequency of spontaneous changes of direction	44.2 ± 11.6	47.7 ± 10.0	52.3 ± 23.0	55.6 ± 19.4*
Time spent in the margin	59.1 ± 6.0	58.1 ± 10.7	58.8 ± 7.4	55.8 ± 8.4
Amount of large movement (%): movement speed >0.10 m/s (% of total observation time)	23.1 ± 4.8	24.1 ± 4.9	23.9 ± 6.5	28.3 ± 8.4*
Amount of local movement (%): movement speed 0.03 m/s < x < 0.10 m/s (% of total observation time)	30.5 ± 2.2	29.5 ± 2.5	30.8 ± 1.8	29.4 ± 2.1
Defecation	1.3 ± 1.7	0.7 ± 0.8	1.1 ± 1.5	2.3 ± 1.8
DBA/2	($n=11$)	($n=16$)	($n=11$)	($n=15$)
Total distance covered (m)	27.9 ± 7.7	34.7 ± 5.6*	28.8 ± 7.5	30.3 ± 7.1
Resting time	60.0 ± 11.9	51.7 ± 3.6**	60.7 ± 6.8	55.1 ± 7.3
Average velocity (m/s)	0.05 ± 0.01	0.06 ± 0.01*	0.05 ± 0.01	0.05 ± 0.01
No of times crossing the center	2.7 ± 3.1	7.8 ± 3.5***	5.6 ± 4.4	4.5 ± 5.3
Frequency of sniffing/rearing	99.2 ± 25.1	99.6 ± 25.5	115 ± 59.2	114 ± 41.3
Frequency of spontaneous changes of direction	29.3 ± 11.4	44.8 ± 11.8**	36.4 ± 17.8	35.7 ± 15.3
Time spent in the margin	77.2 ± 11.3	67.4 ± 8.1**	75.0 ± 11.3	69.7 ± 17.1
Amount of large movement (%): movement speed >0.10 m/s (% of total observation time)	17.1 ± 6.0	23.2 ± 4.0**	16.0 ± 4.1	18.5 ± 5.1
Amount of local movement (%): movement speed 0.03 m/s < x < 0.10 m/s (% of total observation time)	23.0 ± 6.2	25.1 ± 1.9	23.3 ± 3.3	26.4 ± 2.8
Defecation	1.7 ± 1.7	2.3 ± 1.4	1 ± 1.8	2.6 ± 2.2

Asterisks indicate significance of difference of drug-treated mice (C57BL/6J and DBA/2) compared to control group (0.9% NaCl solution), (Student's t-test and/or Mann–Whitney U-test; * $P<0.05$, ** $P<0.01$, *** $P\leq 0.001$)

Table 2c. I. Results of the primary behavioral observation screen in C57BL/6J mice

C57BL/6J	NaCl ($n=12$)	DCS ($n=16$)	SIB1553A ($n=14$)	RS67333 ($n=12$)
A. Behavior				
1. Spontaneous activity				
Body position	5.5 ± 2.3	5.9 ± 2.4	5.5 ± 2.2	6.2 ± 2.0
Locomotor activity	1.8 ± 2.6	2.5 ± 2	2.3 ± 1.5	2.2 ± 1.8
Bizarre behavior	0	0	0	0
2. Motor-affective responses				
Vestibular drop	2.8 ± 1.7	2.9 ± 1.3	1.4 ± 1.4*	1.3 ± 1.1*
Transfer arousal	2.9 ± 1.2	3.4 ± 0.5	3 ± 1.2	3.6 ± 1
Spatial locomotion	4.2 ± 1.4	4.8 ± 1.1	4.4 ± 1.3	5.2 ± 1.2
Touch escape	3.7 ± 1.4	5.8 ± 1.8**	4.8 ± 2.3	4.4 ± 2.0
Provoked biting	3 ± 2	3.6 ± 2.0	2.3 ± 1.3	2.8 ± 2.3
Finger approach	2.8 ± 2.5	2.5 ± 2.1	1.9 ± 2.4	3 ± 1.6
Finger withdrawal	1.5 ± 2.3	1.6 ± 2.2	2.4 ± 2.2	0.8 ± 2
Cliff avoidance	12/0	15/1	14/0	11/1
Vocalization	1.25 ± 1.1	1.8 ± 0.8	1.9 ± 1.4	1.7 ± 1.4
3. Sensor motor response				
Startle response	3.2 ± 2.8	5.3 ± 2.8*	4.1 ± 3.7	2.8 ± 2.9
Pinna reflex	1.5 ± 2.8	1 ± 2.2	0.1 ± 0.5	1.2 ± 2
Cornea	2.3 ± 1.7	2.8 ± 1.2	2.3 ± 1.5	3.2 ± 1.0
Toe pinch	1 ± 1.8	0.3 ± 0.7	0.6 ± 1.5	1 ± 1.3
Visual placing	4.5 ± 1.2	4.1 ± 0.9	3.9 ± 0.9	4.2 ± 1.3
Tail pinch	0.1 ± 0.3	0.5 ± 1.0	0.4 ± 1.1	0.1 ± 0.3
B. Neurology				
1. Posture				
Limb rotation	1.7 ± 0.8	1.1 ± 0.8	1.7 ± 0.8	1.4 ± 0.7
Pelvic elevation	3.3 ± 1.1	4.3 ± 1*	4 ± 1.1	4.6 ± 1.1*
Tail elevation	2.7 ± 0.8	2.5 ± 0.9	2.9 ± 0.9	3.9 ± 0.8***
2. Muscle tone				
Body tone	5.8 ± 1.3	5.8 ± 1.3	6.7 ± 1.4*	7.1 ± 1.0*
Grip strength	4.8 ± 1.8	3.6 ± 1.3*	4.9 ± 2.0	5 ± 1.0
Limb tone	3.3 ± 1.5	4.4 ± 1.6*	3.6 ± 1.6	3.8 ± 2.2
Abdominal tone	5.2 ± 1.8	5.1 ± 1.7	5.3 ± 1.7	5.4 ± 1.1
Wire manoeuvre	2.7 ± 2.3	3.6 ± 2.2	1.6 ± 1.4	2.9 ± 2.4
3. Equilibrium and gait				
Gait	0/12	1/15	0/14	0/12
Righting reflex	0	0	0	0
4. CNS Excitation				
Tremor/twitches	0	0	0	0
C. Autonomic				
1. Eyes				
Palpebral closure	0	0.4 ± 1.5	0	0
Proprioception	5.9 ± 0.7	6 ± 0	6 ± 0	5.6 ± 1.0
2. Secretion and excretion				
Defecation	2.8 ± 1.3	1.9 ± 1.4	2.2 ± 1.4	2.7 ± 1.6
Diarrhea	1/11	0/16	0/14	0/12
Urination	10/2	8/8	2/12*	7/5

Scores (mean ± standard deviation) as evaluated by Irwin et al. (1968): Higher score presents more (better, higher) activity (performance, response) excepting for gait, wire manoeuvre and righting reflex presenting better performance when less score. Some parameters are scored as present: ± (presence/absence)

Table 2c. II. Results of the primary behavioral observation screen in DBA/2 mice

DBA/2	NaCl ($n=11$)	DCS ($n=16$)	SIB1553A ($n=11$)	RS67333 ($n=15$)
A. Behavior				
1. Spontaneous activity				
Body position	5.7 ± 2.2	6.4 ± 1.8	6.3 ± 1.7	6.1 ± 1.9
Locomotor activity	2 ± 1.8	1.5 ± 1.6	2.6 ± 2.0	1.9 ± 1.4
Bizarre behavior				
2. Motor-affective responses				
Vestibular drop	0.7 ± 1.3	1.8 ± 1.3*	0.7 ± 1.1	1.1 ± 1.1
Transfer arousal	2.9 ± 1.3	2.6 ± 1.5	3.4 ± 1.4	3.7 ± 1.3
Spatial locomotion	3.9 ± 1.9	3.7 ± 1.2	4.1 ± 1.1	4.8 ± 1.2
Touch escape	3.8 ± 1.1	4.3 ± 1.9	4.1 ± 2.0	3.9 ± 1.9
Provoked biting	1.5 ± 1.3	2.8 ± 2.3	1.8 ± 1.7	1.7 ± 1.8
Finger approach	2.6 ± 2.5	3.6 ± 2.9	2.6 ± 3.0	3.7 ± 2.6
Finger withdrawal	2.2 ± 2.6	1.3 ± 1.9	2.4 ± 2.9	1.6 ± 2.9
Cliff avoidance	10/1	16/0	11/0	13/2
Vocalization	1.7 ± 0.9	2.4 ± 1.9	2.9 ± 1.6*	1.8 ± 1.4
3. Sensor motor response				
Startle response	2.6 ± 1.8	2.1 ± 2.3	3.7 ± 3.4	3.1 ± 2.8
Pinna reflex	0	0.9 ± 2.1	0.2 ± 0.6	0.8 ± 1.8
Cornea	2.6 ± 1.1	2.6 ± 1.4	2.7 ± 1.0	2.9 ± 1.3
Toe pinch	1.5 ± 2.0	1.3 ± 1.4	1.5 ± 2.4	0.9 ± 1.8
Visual placing	4.4 ± 1.2	4.5 ± 0.9	4.9 ± 1.1	4.7 ± 1.0
Tail pinch	0.9 ± 1.6	1.9 ± 2.8	1.4 ± 2.5	0.7 ± 1.6
B. Neurology				
1. Posture				
Limb rotation	1.3 ± 0.5	1.6 ± 0.5	1.7 ± 0.5*	1.3 ± 0.7
Pelvic elevation	3.5 ± 1.3	3.6 ± 1.1	3.6 ± 1.6	3.2 ± 1.0
Tail elevation	1.8 ± 0.6	2 ± 0.4	2 ± 0	1.8 ± 1.2
2. Muscle tone				
Body tone	6.1 ± 0.3	6.8 ± 0.9*	6.9 ± 1.4	7.1 ± 1.0*
Grip strength	4.4 ± 1.2	5.6 ± 1.6*	4.5 ± 1.8	4.9 ± 2.0
Limb tone	2.7 ± 2.0	3.7 ± 1.7	3.6 ± 1.8	4.2 ± 2.6
Abdominal tone	5.5 ± 1.3	5.8 ± 1.4	6.6 ± 1.6	5.7 ± 1.1
Wire manoeuvre	0.7 ± 1.9	1.8 ± 2.9	1.4 ± 2.5	1.5 ± 2.2
3. Equilibrium and gait				
Gait	0/11	1/15	0/11	0/15
Righting reflex	0	0	0	0
4. CNS Excitation				
Tremor/twitches	0	0	0	0
C. Autonomic				
1. Eyes				
Palpebral closure	0	0	0	0
Proprioception	6 ± 0.8	5.9 ± 0.9	6.3 ± 0.5	6.1 ± 1.0
2. Secretion and excretion				
Diarrhea	2/9	7/9	0/11	9/6*
Urination	2/9	3/13	2/9	2/13
Defecation	2.6 ± 1.7	2.5 ± 1.8	1.7 ± 1.4	2.8 ± 1.8

Scores (mean ± standard deviation) as evaluated by Irwin et al. (1968): Higher score presents more (better, higher) activity (performance, response) excepting for gait, wire manoeuvre and righting reflex presenting better performance when less score. Some parameters are scored as present: ±(presence/absence)

Table 2d. Results of the Rota rod, longest time on the drum was used for analysis

Maximum trial	NaCl	DCS	SIB1553A	RS67333
C57BL	13.3 ± 7.8	14.75 ± 7.8	$17.9 \pm 5.9^{*}$	16.1 ± 9.7
DBA	8.7 ± 7.3	12.6 ± 6.1	13.0 ± 4.6	12.6 ± 5.4

Asterisks indicate significance of difference of drug-treated mice (C57BL/6J and DBA/2) compared to control group (0.9% NaCl solution), (Student's *t*-test and/or Mann–Whitney *U*-test; $^{*}P<0.05$, $^{**}P<0.01$, $^{**}P\leq 0.001$

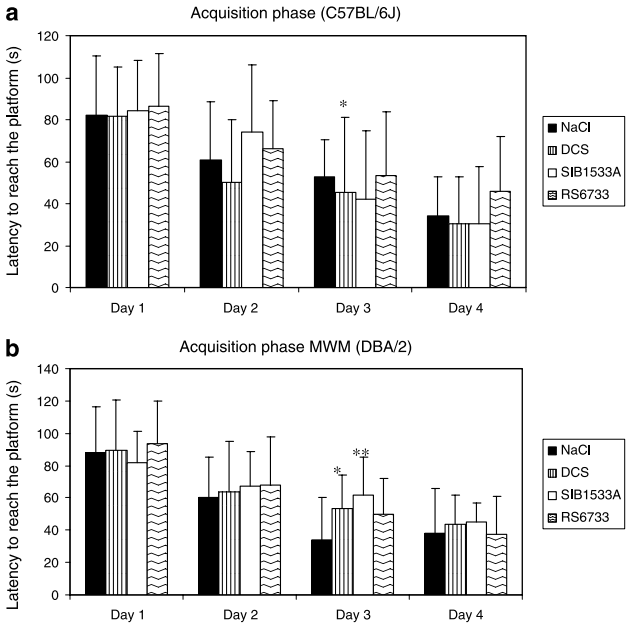

Fig. 1. Escape latency (**a**) in C57BL/6J and (**b**) in DBA/2 mice treated with DCS, SIB-1553A and RS67333 compared with mice treated with vehicle (0.9% NaCl). Asterisks indicate significance of difference of drug-treated mice compared to control group (Student's *t*-test and/or Mann–Whitney *U*-test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P\leq 0.001$)

significant difference between control and treated group on the probe trial.

4. Discussion

The major outcome of the study tells that there are remarkable strain-dependent effects of CEs on behavioral, cognitive and neurological functions. Moreover, evidence for the presence of probable neurotoxic effects was provided.

Observations of increased total distance covered, number of times crossing the centre, frequency of spontaneous changes of direction and amount of large movements may point to hyperactivity that may have been contributing to cognitive decline in DBA/2 mice treated with DCS. Findings in this study are not contradicting the reported

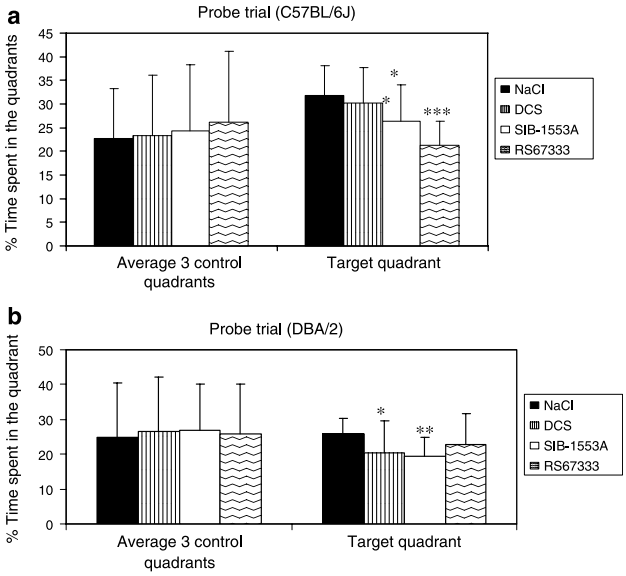

Fig. 2. Percentage of time spent in the target quadrant and average of time spent in 3 control quadrants (**a**) in C57BL/6J and (**b**) in DBA/2 mice treated with DCS, SIB-1553A and RS67333 compared with mice treated with vehicle (0.9% NaCl). Asterisks indicate significance of difference of drug-treated mice compared to control group (Student's *t*-test and/or Mann-Whitney U test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P\leq 0.001$)

CE effects on spatial memory in the mouse per se but failure to improve spatial memory in C57BL/6J clearly indicate that more work is needed before DCS can be assigned an unequivocal CE effect. Behavioral effects in terms of decreased anxiety-related behavior as reflected by OF and EPM (see Table 2a, b) is contradictory to previous findings of anxiogenic effects in low anxiety-bred rats (Ho et al., 2005). The absence of an effect of DCS on spatial memory observed in DBA/2, however, is not contradicting the cognitive enhancing potential either, as there are several underlying causes for failure to increase cognitive performance: DBA/2 mice e.g. present with signaling impairment of reduced protein kinase C in the brain (Wehner et al., 1990) and gene expression profiling showed a large series of differentially expressed genes between C57BL/6J and DBA/2 mice (Kerns et al., 2005).

In the present study C57BL/6J mice performed better than DBA/2 mice in the probe trial in agreement with previous work (Nguyen et al., 2000). On the other hand this finding clearly shows that several strains have to be tested when a CE is being evaluated.

Decades ago DCS was described as a potential cognitive enhancer improving spatial memory in the rodent. In Sprague-Dawley rats intra-peritoneal DCS, administered daily, increased acquisition in the MWM (Lelong et al, 2001), decreased latency in a water maze in aging 4 MO

rats (Baxter et al., 1994) and decreased number of errors in a radial arm maze task in adult Wistar rats (Pussinen and Sirvio, 1999). Aura and coworkers showed that intraperitoneal administration of DCS enhanced acquisition in aged but not young Han:Wistar rats in a MWM task (Aura et al., 1998). Several doses failed to enhance acquisition and memory performance in male Han:Wistar rats using the MWM paradigm (Pitkänen et al., 1995). Therefore, no consistent CE effect of DCS was observed and that may well be due to different rat strains, ages, test systems.

In mice, post-training administration of DCS significantly increased memory retention in a thirst-motivated linear maze in Swiss-Webster mice (Quartermain et al., 1994) but no other strains or test systems were used so far. In the present study no effect on spatial memory in the MWM was observed for C57BL/6J. In DBA/2, however, consolidation expressed as decreased time spent in the target quadrant of the MWM was impaired and the learning curve was indicating disturbed acquisition, at least at the third day of training.

Behavioral or neurological side effects may not be incriminated for the poor performance in the MWM in DBA/2 mice and again, underlying neuropathology or strain-differences may have been responsible for failure of DCS treatment.

SIB-1553A treatment in both strains did not alter performance in OF and EPM. However, increased body tone and remarkably altered vestibular drop in the neurological observational battery was observed in C57BL/6J treated mice. On the other hand, excellent performance on the Rota rod is relativizing the (non-quantitative) findings from the observational battery. The clear effect of SIB-1553A on improving motor coordination and motor function is a new finding that may have potential therapeutic relevance and this will be further investigated in our laboratory. Subcutaneous administration of SIB-1553A to aged C57BL/6J mice induced a dose-dependent decrease in locomotor activity and significantly impaired motor coordination of young mice at high doses (146 μmol/kg) (Bontempi et al., 2003), but at the dose used in our experiments, 2.5 mg/kg, an about 25% improvement of Rota-rod performance was noticed.

Administration of SIB-1553A in a primate (rhesus monkeys) led to improvement of correct decisions in the delayed and non-delayed matching-to-sample task. In the Fisher rat, SIB-1553A did not improve acquisition in the MWM but enhanced consolidation as evaluated by an increased time spent in the target quadrant. In the mouse system, the effect of SIB-1553A treatment was tested in a T-maze in the form of a two-arm discrimination task and improvement of correct responses in aged C57BL/6J mice was revealed; unfortunately, young mice were not tested (Bontempi et al., 2003). In the present study SIB-1553A had no effect on acquisition and consolidation was significantly impaired in both strains.

Also RS67333 was tested in primates (macaques) in a delayed matching task and an elevated percentage of correct decisions was observed in young and old animals (Terry et al., 1998). Fontana et al. (1997) showed reversion of atropine-induced cognitive decline in the MWM following intraperitonial injection of RS67333 to Sprague-Dawley rats. Lelong (2001) studied the effect of RS67333 in Sprague-Dawley rats and observed increased acquisition in the MWM. In a place recognition task, Orsetti et al. (2003) showed increased acquisition and consolidation in male Wistar rats following RS67333 administration into the nucleus basalis magnocellularis. In the mouse system, the only study was carried out by Lelong et al. (2003) showing that RS67333 prevented scopolamine-induced alteration deficits in male NMRI mice.

Herein, RS67333 impaired consolidation in C57BL/6J but not in DBA/2. Again decreased vestibular drop may have been affecting the outcome in the MWM. Findings in the OF in C57BL/6J could be interpreted as increased activity as reflected by increased distance covered, elevated speed, amount of large movements whereas increased centre crossings and frequent spontaneous change of direction would be compatible with anxiolytic activity. Tail and pelvic elevation as well as increased body tone observed in the observational battery would be in agreement with increased activity as well. No behavioral effects would represent confounding factors for the evaluation of cognitive functions in DBA/2 mice and the role of the single behavioral finding of diarrhea in RS67333-treated animals remains open.

Taken together, we learn from the study that CE effects may be strain-dependent and it is therefore proposed that several strains have to be tested to finally make a reliable statement on cognitive enhancement per se. Animals with known neurochemical defects as e.g. DBA/2, although widely used in cognitive research, should not be included in cognitive protocols, and pre-evaluation of neurological and behavioral function may help to select experimental mouse strains or even sub-strains. A main claim for performing behavioral and neurological tests along with cognitive evaluation has to be made to enable fair interpretation of cognitive results. And indeed, CEs have been presenting with several behavioral and neurological side effects. These side effects are not simply adverse effects but show that anxiety-related behavior as well as motor

coordination and activity can be modulated by CEs. A major inherent problem in comparing results with literature is that many different tasks and protocols for the evaluation of acquisition and consolidations for spatial memory were used and the generation and use of strictly evaluated protocols suggested by the neuroscientifique forum are recommended.

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