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α_{2C}-Adrenoceptor-Overexpressing Mice Are Impaired in Executing Nonspatial and Spatial Escape Strategies

MARKUS BJÖRKLUND, JOUNI SIRVIÖ, JUKKA PUOLIVÄLI, JUKKA SALLINEN, PEKKA JÄKÄLÄ, MIKA SCHEININ, BRIAN K. KOBILKA, and PAAVO RIEKKINEN, JR.

Department of Neurology and Neuroscience, University of Kuopio, Kuopio, FIN-70211, Finland (M.B., J.P., P.J., P.R.), A. I. Virtanen Institute, FIN-70211 Kuopio, Finland (J. Si.), Department of Pharmacology and Clinical Pharmacology, University of Turku, FIN-20520 Turku, Finland (J. Sa., M.S.), Kuopio University Hospital, FIN-70211 Kuopio, Finland (P.J., P.R.), and Department of Molecular and Cellular Physiology, Howard Hughes Medical Institute and Division of Cardiovascular Medicine, Stanford University, Stanford, California 94305 (R.K.K.)

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ABSTRACT

Drugs acting via α_2-adrenoceptors modulate cognitive functions mediated via frontostriatothalamic feedback loops. The α_{2C}-adrenoceptor subtype is expressed in the basal ganglia, hippocampus, and neocortex, areas that are involved in memory and other cognitive functions. α_{2C}-Overexpressing (OE) mice were impaired in spatial or nonspatial water maze (WM) tests, and α_2 antagonist treatment fully reversed the WM escape defect in OE mice. However, α_{2C}-overexpression did not influence open field and passive avoidance behaviors or cortical EEG arousal or the actions of α_2 agonist or antagonist drugs on these functions. Our results suggest that α_{2C}-adrenoceptors can modulate navigation to a hidden or visible escape platform, whereas many other actions of α_2-adrenergic agents, such as sedation, are not mediated via α_{2C}-adrenoceptors. Therefore, α_2-agonists lacking α_{2C}-AR affinity or α_{2C}-AR subtype-selective α_2 antagonists could modulate functioning of frontostriatothalamic feedback loops more effectively than the current subtype-nonselective drugs.

LC norepinephrine neurons send noradrenergic fibers into different forebrain structures (Fillenz, 1990) and modulate different cognitive functions, such as arousal, attention, and planning (Crow, 1968; Kety, 1970; Arnsten and Goldman-Rakic, 1985; Arnsten and Leslie, 1991; Riekkinen *et al*., 1992; Sahakian *et al*., 1994; Arnsten *et al*., 1996; Coull *et al*., 1996). Because ARs are located both presynaptically and postsynaptically, it is not surprising that pharmacological studies have found that noradrenergic α_2-AR agonists and antagonists can affect many behaviors mediated by different neural systems. α_2-ARs are divided into three different subtypes, termed α_{2A}-, α_{2B}-, and α_{2C}-ARs (Kobilka *et al*., 1987; Regan *et al*., 1988; Lomasney *et al*., 1990; Link *et al*., 1992), and all these subtypes have distinct anatomic distributions in brain areas involved in separate functional systems (Nicholas *et al*., 1993, 1996; Aoki *et al*., 1994; Scheinin *et al*., 1994; MacDonald and Scheinin, 1995; Rosin *et al*., 1996; Talley *et al*., 1996). α_{2A}-ARs are located in the LC, elsewhere in the brainstem, and throughout the cerebral cortex and many deeper forebrain structures; α_{2B}-ARs are found nearly exclusively in the thalamic nuclei; and α_{2C}-ARs are located in the hippocampus, cerebral cortex, and striatum. Importantly, in the caudate and accumbens nuclei, α_{2C}-ARs predominate, suggesting that this receptor subtype may mediate effects of α_2 agonists and antagonists on modulation of cognitive functions via frontostriatothalamic feedback loops.

The behavioral functions of different subtypes of α_2-ARs have been difficult to study because there are no ligands that selectively activate or block only one of the three subtypes. Therefore, to study the role of different α_2-ARs in behavioral functions and to better evaluate the potential for cognition-enhancement by subtype-selective α_2-AR active drugs, we have started to investigate the effects of overexpression and knockout of α_2-AR subtype genes on different behaviors in mice and how these manipulations affect reactions to subtype-nonselective α_2-AR agonists and antagonists (MacDonald *et al*., 1997). Sallinen *et al*. (1997) found that spontaneous locomotor activity and the effects of an α_2-agonist or -antagonist on brain monoamine turnover and on motor activity were not influenced by altered α_{2C}-AR expression. However, mice with tissue-specific overexpression of α_{2C}-AR were slightly more sensitive and KO mice were less sensitive to the hypothermic effects of the agonist DEX than their controls. The OE mice have ~3-fold elevated densities of α_{2C}-AR in regions that normally express this subtype (the

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ABBREVIATIONS: AR, adrenoceptor; ATI, atipamezole; CKO, wild-type control mice for α_{2C}-knockout mice; DEX, dexmedetomidine; EEG, electroencephalogram; KO, α_{2C}-knockout; LC, locus ceruleus; OE, α_{2C}-overexpressing; OF, open field; PA, passive avoidance; WM; water maze; WT, wild-type control mice for α_{2C}-overexpressing mice

caudate-putamen and CA1 region of the hippocampus) (Sallinen *et al.*, 1997). In KO mice, the total α_2-AR density is diminished in brain areas normally expressing the α_{2C} subtype (Link *et al.*, 1995). In this study, cue and spatial WM navigation were evaluated in OE mice and control (WT) mice and spatial navigation was evaluated in KO mice and CKO because our previous studies have shown that α_2-AR agonists may modulate performance in these tests (Sirviö *et al.*, 1991, 1992). Furthermore, to study the specificity of the WM navigation changes observed in OE mice, we also compared behaviors of WT and OE mice in OF and PA tests (Riekkinen *et al.*, 1992). In addition, cortical EEGs were investigated. All these tests are modulated by α_2-adrenergic drugs (Riekkinen *et al.*, 1990, 1993). The OF test is a measure of anxiety; thus if animals are anxious, they will spend more time in the peripheral annulus of the OF arena. In the WM task, the escape platform is located in the middle annulus, and anxiety-induced swimming in the peripheral annulus reduces the chances to find the platform. The PA test measures stimulus-response learning. We aimed to test whether OE mice have a learning defect in the PA paradigm. Cortical EEG monitoring was performed to detect differences in general arousal. An appropriate state of arousal is needed to perform accurately in the WM test. We also studied whether OE mice have an altered response to the sedative action of α_2-adrenergic drugs. The effects of α_{2C}-AR overexpression may be due to an increase in basal and agonist-activated signal transduction mediated through this receptor subtype. Elevated levels of receptor expression can lead to an increase in basal, constitutive signal transduction, which can mimic agonist activation (Samama *et al.*, 1997). To examine the role of enhanced signaling of α_{2C}-AR on the defect in WM performance in OE and WT mice, we examined the effect of ATI, a highly α_2-AR-specific but not subtype-selective antagonist. Furthermore, to assess the role of α_{2C}-ARs in the sedative actions of α_2-AR agonists in WT and OE mice, we also compared the effect of DEX, a highly specific α_2-AR agonist that does not differentiate between α_2-AR subtypes. There has been no rigorous evaluation of ectopic α_{2C} expression in brain regions outside those normally expressing α_{2C}-AR. Such ectopic expression could account for the differences seen in OE and WT mice in their WM behavior. To study the problem of ectopic expression, we used KO mice in a control WM study.

Materials and Methods

Animals

Adult (3–5 months old;: weight, 20–28 g) female FVB/N (control mice for OE mice, WT) and FVB/N-TgNAdra2C (OE) (both OE and WT mice were from Stanford University Medical Center, Stanford, CA) were used in the study (total, 109 OE and 106 WT mice). This strain of genetically engineered mice, which has tissue-specific overexpression of α_{2C}-ARs, was generated at Stanford University by pronuclear injection using standard methods. OE mice have ~3-fold elevated densities of α_{2C}-AR in their caudate-putamen and CA1 region of the hippocampus (Sallinen *et al.*, 1997).

Adult (3–5 months old; weight, 20–28 g) female C57BL/6J for CKO and KO mice also were used in the study (total, 34 KO and 33 CKO mice). A strain of genetically engineered mice that had targeted inactivation of the α_{2C}-AR gene was generated at Stanford University (both KO and CKO mice were from Stanford University Medical Center). KO mice do not express a functional α_{2C} transcript (Link *et al.*, 1995).

All the mice used in the current experiments were bred in the Central Laboratory Animal Facility of the University of Turku, Finland, according to standard procedures. The mice were housed five per cage. The housing conditions (National Animal Center, Kuopio, Finland) were controlled; constant temperature (21 ± 1°), humidity (50–60%), and light period (lights on from 7:00 a.m. to 7:00 p.m.) were maintained; and food and fresh water were freely available. All experiments were performed during the light period. The study was approved by the Animal Welfare Committee of the University of Kuopio.

Drugs

ATI (3–300 μg/kg; Orion Corporation Farmos, R & D Pharmaceuticals, Turku, Finland), a selective α_2 antagonist (Scheinin *et al.*, 1988; Virtanen *et al.*, 1989), and DEX (0.5–300 μg/kg; Orion Corporation Farmos, R & D Pharmaceuticals), a selective α_2 agonist (MacDonald *et al.*, 1991; Savola and Virtanen, 1991), were dissolved in saline and injected subcutaneously (5 ml/kg). ATI and DEX were injected 20 min before daily behavioral testing, and for four WM groups, ATI (30–300 μg/kg) was administered immediately after daily training.

WM

Cue and spatial navigation were evaluated in a WM pool (black; diameter, 59 cm). Four starting points (north, south, east, and west) were located at the pool rims. A black 3.5- × 3.5-cm platform was located in the middle annulus, 0.5 cm above (visible) or 1 cm below (hidden) the water line. The visible platform had a 10-cm-high white mast. The location of the visible platform was changed daily during the visible platform training (cue navigation), but the hidden platform (place navigation) was kept in a fixed place. The mice were facing the wall and were gently released to begin the first daily trial from the starting position farthest from the platform. The other four trials were started in a semirandom order. Five trials with a cutoff value of 50 sec were tested every day (5-sec reinforcement on the platform). The mice that did not find the platform were placed on it for 5 sec. A 30-sec recovery period was allowed between the trials. A computerized video monitoring system (HVS Image, Hampton, UK) calculated the number of animals that failed to find the platform, swimming speed, and swimming in three annuli of equal surface area. Our preliminary data showed that the KO mice and their controls performed better than OE mice and their controls in the WM task. The KO and CKO mice completed the task (i.e., they found the platform) almost every time. Therefore, the parameter "platform finding" (completing the task) was too insensitive to detect any difference between CKO and KO mice, so we applied here a widely used measure, swimming distance (i.e., the distance the animals swam before completing the task or reaching the cutoff time value).

Experiment 1. We used the following treatments for both the WT and the OE mice: saline, ATI 30 and 300 μg/kg (15 OE or WT mice/group). First, we trained the mice to find a visible platform (cue navigation) that was moved every day to a novel position. Training continued for 5 consequent days (five trials/day as described above).

Next, we trained the mice for 2 days without drug treatment to find a visible platform that was moved every day to a new location. After this, the mice were trained to find a hidden platform (spatial version of the WM task). The platform was kept in the same position throughout the 5-day training period. Daily sessions were similar to the visible platform version.

Experiment 2. The following treatments were used for both the WT and the OE mice: saline, ATI 30 and 300 μg/kg immediately after daily training (11–13 OE or WT mice/group). These mice had only 5 days of hidden platform training.

Experiment 3. The following treatments were used for both the CKO and the KO mice: saline, DEX 0.5 and 10 μg/kg (10–13 KO or CKO mice/group). These mice had only 3 days of hidden platform training.

Cortical EEG measurements

Two stainless steel screws acting as epidural recording electrodes were bilaterally implanted 1.0 mm anterior and 2.0 mm lateral to bregma. Two additional screws acting as indifferent and ground electrodes were implanted in the nasal bone and above the cerebellum. The effects of DEX (3–300 μg/kg) and ATI (3–300 μg/kg) on cortical EEG activity were measured by recording five 4-sec-long artifact-free EEG episodes with relaxed waking-immobile animals (24 OE and 22 WT). EEG spectra were divided into the frequency bands of 1–4 Hz δ, 4–8 Hz θ, 8–12 Hz α, 12–20 Hz β, and two upper frequency bands of 20–30 and 30–60 Hz.

OF

A dry Morris WM pool was used to perform an OF task. The mice were placed on the bottom of the pool with the nose pointing toward the wall. A computer connected to an image analyzer calculated the total walking distance, walking speed, and time spent in the three annuli. Effects of SAL and DEX and ATI were tested. One 50-sec trial of free exploration was given to all mice.

PA

A single training trial step through PA test was conducted after the WM study. In the training trial, the experimenter injected the mice with SAL, DEX (2.0, 5.0, or 20.0 μg/kg), or ATI (30 or 300 μg/kg); 20 min later, the mice were placed at the far end of the bright side of the PA box and the guillotine door was opened 30 sec later. The latency to enter the dark side was measured. Ten seconds after the entry (all four paws inside), an electric shock (DC current 0.20 mA, 3-sec duration; Shock Source 521 C, Campden Instruments, Leicestershire, UK) was given. After 24 hr, the mouse was placed again on the bright side, and 30 sec later, the guillotine door opened (testing trial; maximum testing time of 360 sec as a cutoff value). The reentry latency was measured.

Statistics

The effects of strain, drug treatment, and their interaction (strain × treatment) were evaluated using analysis of variance for repeated measurements and simple factorial analysis of variance.

Results

WM experiment 1: ATI 30 and 300 μg/kg before daily training

Effects of α_{2C} overexpression on WM escape behavior. *Visible platform days 1–5*. Compared with WT mice, OE mice did not find the visible platform as accurately [strain: $F(1,28) = 19.251$, $p < 0.001$] (Fig. 1A). During visible platform training, the swim pattern of OE mice was not different from WT mice as they swam equally in the peripheral, middle, and inner annuli [strain: $F(1,28) \leq 4.11$, $p \geq 0.052$, for all].

Visible platform days 6–7. OE mice did not navigate to the visible platform as accurately as WT mice [strain: $F(1,28) = 19.14$, $p < 0.001$] (Fig. 1B). Compared with WT mice, OE mice swam more in peripheral [strain: $F(1,28) = 15.11$, $p = 0.001$], equally in middle [strain: $F(1,28) = 1.68$, $p > 0.1$], and less in the inner [strain: $F(1,28) = 11.91$, $p = 0.002$] annulus.

Hidden platform days 8–12. During hidden platform training, OE mice navigated to the platform as accurately as WT mice [strain: $F(1,27) = 1.801$, $p > 0.1$] (Fig. 1C). OE mice swam more in the peripheral but less in the middle and inner annuli [strain: $F(1,27) \geq 5.30$, $p \leq 0.03$, for all].

OE mice swam at a slower speed than WT mice [strain: $F(1,27) \geq 5.14$, $p \leq 0.032$, for all stages] during all of the training stages (Table 1).

Effects of ATI 30 μg/kg on WM escape behavior. *Visible platform.* ATI 30 μg/kg only tended to improve platform finding in WT and OE mice [treatment: $F(1,56) = 3.737$, $p = 0.058$] (Fig. 1A). ATI 30 μg/kg decreased swimming in the peripheral annulus [treatment: $F(1,56) = 14.99$, $p = 0.001$]. ATI 30 μg/kg increased swimming in the middle annulus, which contained the platform, more effectively in OE mice than in WT mice [treatment: $F(1,56) = 14.14$, $p = 0.001$, strain × treatment: $F(1,56) = 7.40$, $p = 0.009$). ATI 30 μg/kg did not affect swimming in the inner annulus [treatment: $F(1,56) = 2.97$, $p > 0.05$].

Visible platform days 6–7/ATI 30 μg/kg discontinued. The WT and OE mice that were treated with ATI 30 μg/kg on days 1–5 did not differ in swim speed, escape distance, and swimming in the three different annuli significantly from their controls [treatment: $F(1,56) \leq 3.9$, $p > 0.05$, for all] (Fig. 1B).

Hidden platform days 8–12. ATI 30 μg/kg treatment increased platform finding in WT mice but was ineffective in OE mice [treatment: $F(1,54) = 14.137$, $p = 0.001$; strain × treatment: $F(1,54) = 6.819$, $p = 0.012$] (Fig. 1C). ATI 30 μg/kg decreased swimming in the peripheral annulus and increased swimming in the middle annulus [treatment: $F(1,56) \geq 4.69$, $p < 0.05$, for both]. ATI 30 μg/kg increased swimming in the inner annulus in WT mice but not in OE mice [treatment: $F(1,56) = 9.32$, $p = 0.003$; strain × treatment: $F(1,56) = 4.13$, $p = 0.047$].

ATI 30 μg/kg had no effect on swim speed [treatment: $F(1,56) < 1.19$, $p > 0.1$, for visible and hidden platform training; Table 1].

Effects of ATI 300 μg/kg on WM escape behavior. *Visible platform.* ATI 300 μg/kg improved visible platform finding in OE mice more effectively than in WT mice [treatment: $F(1,56) = 14.022$, $p = 0.001$; strain × treatment: $F(1,56) = 5.548$, $p = 0.022$] (Fig. 2A). ATI 300 μg/kg decreased swimming in the peripheral annulus equally in WT and OE mice [treatment: $F(1,56) = 32.16$, $p < 0.001$; strain × treatment: $F(1,56) = 0.61$, $p > 0.1$]. ATI 300 μg/kg did not affect swimming in the middle annulus [treatment: $F(1,56) = 3.58$, $p = 0.064$] but increased swimming in the inner annulus equally in OE and WT mice [treatment: $F(1,56) = 18.47$, $p < 0.001$].

Visible platform days 6–7/ATI 300 μg/kg discontinued. Both OE and WT mice that had previously received ATI 300 μg/kg navigated to the visible platform better than their controls [treatment: $F(1,55) = 8.742$, $p = 0.005$; WT versus OE with previous ATI treatment: $F(1,27) = 9.865$, $p = 0.004$] (Fig. 2B). Previous treatment with ATI 300 μg/kg did not affect swimming in peripheral, middle, or inner annuli [treatment: $F(1,55) < 1.62$, $p > 0.1$, for all].

Hidden platform days 8–12. ATI 300 μg/kg treatment increased platform finding equally in WT and OE mice [treatment: $F(1,52) = 30.832$, $p < 0.001$] (Fig. 2C). ATI 300 μg/kg decreased swimming in the peripheral annulus and correspondingly increased it in the inner annulus, only in WT mice [treatment: $F(1,55) \geq 13.19$, $p \leq 0.001$; strain × treatment: $F(1,55) \geq 4.82$, $p \leq 0.03$, for both]. ATI 300 μg/kg significantly increased swimming in the middle annulus in OE mice (OE vehicle versus OE ATI 300 μg/kg: $F(1, 28) = 6.05$, $p = 0.020$] but was ineffective in WT (WT vehicle versus WT ATI

300 μg/kg: $F(1,27) = 0.18$, $p > 0.1$] mice [treatment: $F(1,55) = 1.39$, $p > 0.1$; strain × treatment: $F(1,55) = 3.44$, $p = 0.069$].

ATI 300 μg/kg decreased swimming speed during visible platform training [treatment: $F(1,56) = 6.26$, $p = 0.015$] but not during hidden platform training [treatment: $F(1,52) = 0.11$, $p > 0.1$] (Table 1).

WM experiment 2: ATI 30–300 μg/kg after daily training

Effects of ATI 30–300 μg/kg after daily training on WM escape behavior. *Hidden platform days 1–5.* ATI 30–300 μg/kg after daily training had no effect on platform finding, swimming in the peripheral, middle or inner annuli, or swim speed [treatment: $F(1,44) \leq 1.79$, $p > 0.1$, for all] (data not shown).

WM experiment 3: KO DEX 0.5–10 μg/kg before daily training

Effects of KO on WM escape behavior. KO mice found the hidden platform as accurately as CKO mice [strain: $F(1,23) = 2.065$, $p > 0.1$]. The swimming distance of KO and CKO mice was equal [strain: $F(1,23) = 2.53$, $p > 0.1$] (Fig. 3). During hidden platform training, the swim pattern of KO mice was not different from CKO mice as they swam equally in the peripheral, middle, and inner annuli [strain: $F(1,23) \leq 1.42$, $p > 0.1$, for all].

Effects of DEX 0.5–10 μg/kg on WM escape behavior. DEX 10 μg/kg increased swimming distance more effectively in CKO than in KO mice [treatment: $F(1,43) = 22.47$, $p < 0.001$; strain × treatment: $F(1,43) = 4.18$, $p = 0.047$] (Fig. 3). DEX 10 μg/kg had no effect on the other parameters mea-

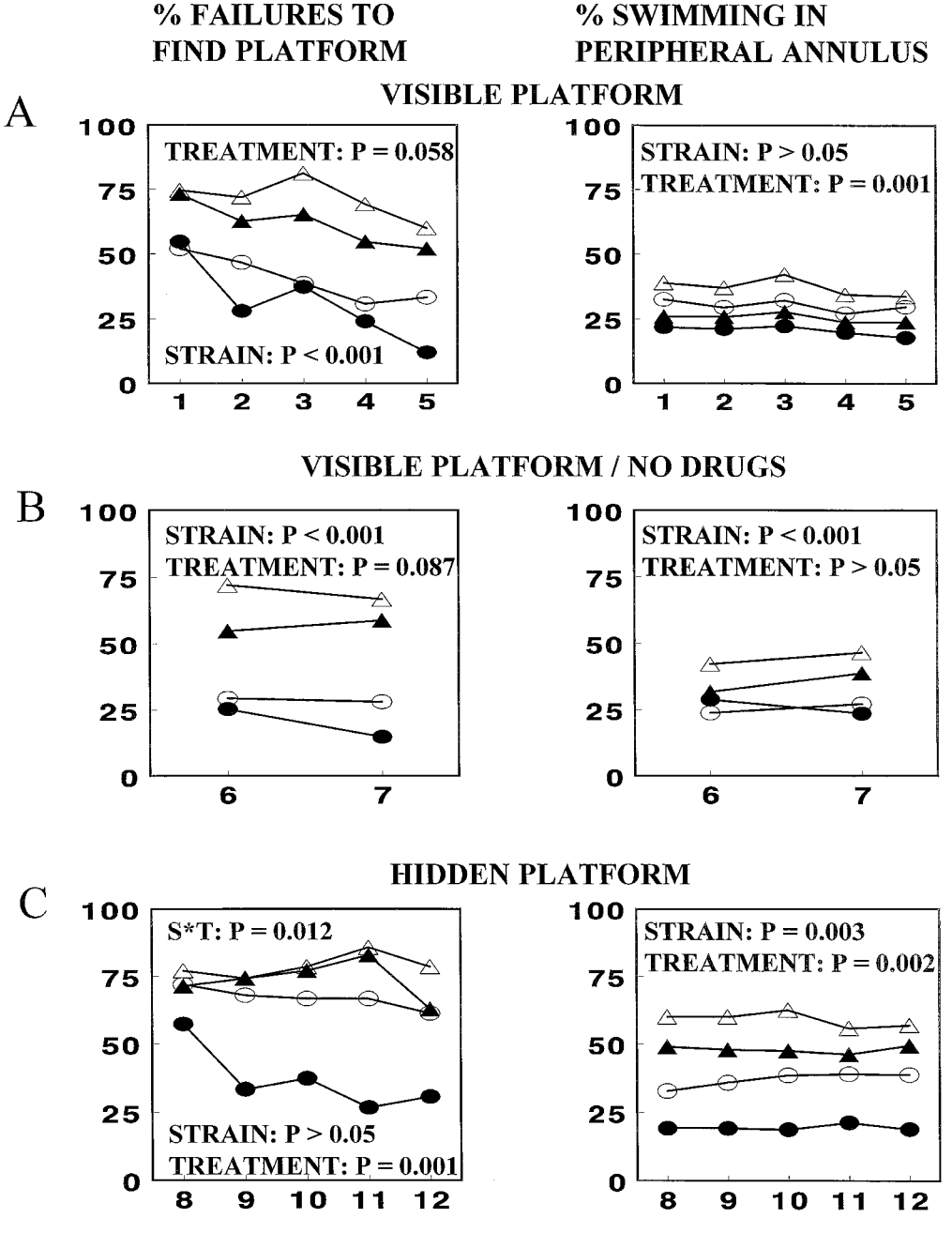

Fig. 1. Mice overexpressing (*OE*) α_{2C}-AR were impaired compared with the control (WT) mice in WM escape performance: fewer OE mice found the platform (percent failures to find platform), and the time spent in the peripheral annulus, which did not contain the platform, was higher (percent swimming in peripheral annulus). The escape platform was located in the middle annulus. The effects of ATI 30 μg/kg are also shown. *Left*, percentage of mice that failed to find the platform (*y*-axis), *Right*, time spent in the peripheral annulus (*y*-axis). The *x*-axis shows the training day. Daily group mean values are shown. The *p* values of significant strain and treatment effects and strain × treatment (*S* × *T*) interactions are shown. ○, WT saline; △, OE saline; ●, WT ATI 30 μg/kg; ▲, OE ATI 30 μg/kg. A, Atipamezole (ATI; 30 μg/kg subcutaneous) stimulated accuracy of WM visible platform (place navigation) navigation and decreased swimming in the peripheral annulus in WT and OE mice. B, During drug-free training days, control and previously ATI 30 μg/kg-treated mice performed equally. C, ATI 30 μg/kg was not as effective at improving platform finding in OE mice during the hidden platform (spatial navigation) test but stimulated accuracy of WT mice. However, ATI 30 μg/kg decreased swimming in the peripheral annulus equally effectively in both strains.

sured [treatment: $F(1,43) \leq 1.86$, $p > 0.1$, for all]. DEX 0.5 μg/kg had no effect on any of the parameters measured [treatment: $F(1,41) \leq 1.93$, $p > 0.1$, for all].

Cortical EEG measurements

WT and OE mice showed no difference in delta amplitudes measured during base-line recordings [strain: $F(1,44) = 1.82$, $p > 0.1$]. DEX 3–300 μg/kg increased delta amplitude in WT and OE mice [treatment: $F(1,44) \geq 5.22$, $p < 0.05$, for all] (Fig. 4A). ATI 3 μg/kg decreased delta amplitude [treatment: $F(1,44) = 5.15$, $p = 0.028$]. ATI 30–300 μg/kg had no effect on delta amplitude [treatment: $F(1,44) \leq 2.77$, $p > 0.05$].

OF

OE and WT mice had similar walking speed and explored equally the peripheral, middle, and inner annuli [strain: $F(1,18) \leq 3.706$, $p \geq 0.071$, for all]. DEX 0.5–5 μg/kg and ATI 30–300 μg/kg had no effect on walking speed or ambulation in the peripheral, middle, and inner annuli [treatment: $F(1,32) \leq 3.1$, $p > 0.05$, for all] (Fig. 4B).

Passive avoidance, DEX 2–20 μg/kg and ATI 30–300 μg/kg

The training and testing latencies of WT and OE mice did not differ [strain: $F(1,27) \leq 3.8$, $p > 0.05$, for both] (Fig. 4, C and D).

Training. ATI 30–300 μg/kg had no effect on training latency [treatment: $F(1,53) \leq 1.598$, $p > 0.1$] (data not shown). DEX 2–20 μg/kg increased training latency [treatment: $F(1,34) \geq 6.080$, $p \leq 0.002$] (Fig. 4C).

Testing. ATI 30–300 μg/kg had no effect on testing latency [treatment: $F(1,53) \leq 0.889$, $p > 0.1$] (data not shown). DEX 2–5 μg/kg had no effect on testing latency [treatment: $F(1,34/1,35) \leq 2.038$, $p > 0.1$]. DEX 20 μg/kg decreased testing latency in both WT and OE mice [treatment: $F(1,34) = 23.319$, $p < 0.001$] (Fig. 4D).

TABLE 1

Swimming speed of different treatment groups during visible and hidden platform training days.

OE mice swam slower than WT mice during all of the training stages. ATI 30 μg/kg had no effect on swimming speed. ATI 300 μg/kg decreased swimming speed during visible platform training but not during hidden platform training.

DEX 0.5–2 μg/kg had no effect on swimming speed, whereas DEX 5 μg/kg decreased swimming speed. Mean ± standard deviation values for 5 training days are shown.

Group	Visible platform training	Hidden platform training
	cm/sec	
WT saline (ATI)	17.47 ± 1.97	17.00 ± 3.40
OE saline (ATI)	13.96 ± 2.69[b]	14.12 ± 3.44[b]
WT ATI 30	17.84 ± 2.09	19.62 ± 2.42
OE ATI 30	12.21 ± 2.95	12.33 ± 3.99
WT ATI 300	15.27 ± 3.56[a]	17.19 ± 3.46
OE ATI 300	12.52 ± 2.82	14.58 ± 3.91
WT saline (DEX)	20.94 ± 2.39	21.66 ± 2.39
OE saline (DEX)	16.71 ± 1.78[b]	17.97 ± 1.90†
WT DEX 0.5	20.20 ± 1.66	20.85 ± 2.44
OE DEX 0.5	17.00 ± 1.65	18.30 ± 3.48
WT DEX 2	20.43 ± 2.36	21.73 ± 2.44
OE DEX 2	16.56 ± 3.22	19.49 ± 3.13
WT DEX 5	18.24 ± 1.35[a]	18.87 ± 1.60[a]
OE DEX 5	15.41 ± 1.69	17.03 ± 2.90

[a] $p < 0.05$ vs. saline-treated own control (WT or OE) mice.
[b] $p < 0.05$ vs. saline-treated WT control mice.

Discussion

OE mice developed an abnormal escape pattern (Simon *et al.*, 1994), characterized by increased swimming in the peripheral annulus of the pool (near the walls), and could not find the visible or hidden platform as accurately as the WT mice. This defective exploration pattern is likely to result from α_{2C}-AR overexpression because a dose-response relationship for ATI was observed. First, we observed that ATI 30 μg/kg slightly decreased swimming near pool walls during hidden and visible platform finding in both groups, but the OE mice were impaired compared with the WT mice at this dose. In contrast, ATI 300 μg/kg fully blocked the abnormal search pattern in OE mice. Second, the treatment with ATI 30 μg/kg did not stimulate accuracy to find the platform during the visible platform stage in OE or WT mice, but during the hidden platform stage, ATI 30 μg/kg clearly and exclusively stimulated the accuracy of WT mice. This suggests that the low dose of ATI 30 μg/kg is not sufficient to overcome the increased α_{2C}-AR function in OE mice, but in WT mice it is able to effectively antagonize α_{2C}-AR function and improve accuracy of WM escape behavior. In contrast, a higher dose of the α_2-AR antagonist ATI 300 μg/kg may block α_{2C} AR function in both WT and OE mice and thus improve WM navigation. The high dose of ATI 300 μg/kg improved visible platform finding of OE mice to equal accuracy as that of WT mice but had no further effect in WT mice. This finding that ATI stimulates visible platform navigation only in OE mice indicates that the α_{2C}-AR may play a more crucial role in the control of those brain areas important for cue navigation than the other α_2-AR subtypes.

We evaluated the WM performance using different versions of WM training: visible platform, visible platform without drug treatment, and hidden platform. The strategies used to locate a visible platform are different from those needed for hidden platform navigation. Visible platform training is a nonspatial version of the WM task. During visible platform training, animals are required to seek the clearly visible escape platform without the need to use distal extra-maze cues. The use of extra-maze cues is disruptive during visible platform training because the platform location is changed daily. During the hidden platform training, the use of extra-maze cues is unavoidable because no intramaze cues are present. The withdrawal of drug treatment at the end of the visible platform training phase was aimed at investigating whether the drug treatments had any long lasting effects on memory of WM performance. The present findings suggest that the α_{2C}-AR overexpression does not impair and ATI does not stimulate cue and spatial navigation by affecting memory processes *per se*. First, the OE mice were impaired already during the first day of training, the learning curves of WT and OE mice remained parallel during the experiment, and ATI failed to modulate the slope of the learning curves. Traditionally, parallel learning curves have been related to defects in processes other than learning and memory. Furthermore, we observed that administration of ATI 30 or 300 μg/kg immediately after daily training trials had no effect on performance, thereby ruling out any beneficial effects on memory consolidation processes. Finally, during the drug withdrawal training period on days 6 and 7, the performance of mice treated with ATI during the training days 1–5 was superior to that of saline-treated WT and OE

mice. During days 1–5, no difference existed in the escape ability of WT and OE mice receiving daily ATI 300 μg/kg treatment, but discontinuation of ATI on days 6 and 7 released functioning of overexpressed α_{2C}-ARs and disturbed the accuracy of OE mice. Therefore, it is possible that α_{2C}-AR overexpression disrupts the accuracy of WM navigation by inhibiting the execution of the normal escape behavior required for success in the WM paradigm and releases abnormal exploration patterns. However, because OE mice treated with ATI 300 μg/kg performed better during the drug withdrawal stage than the vehicle-treated OE mice, it is possible that α_{2C}-ARs may also, to some extent, control memory acquisition of cue navigation performance.

Previous studies describing the distribution of α_{2C}-ARs and the anatomic pattern of α_{2C}-AR overexpression (Sallinen *et al.*, 1997) suggest that the striatum and CA1 region of the hippocampus may represent regions that are under increased α_{2C}-AR modulation in our OE mouse strain. Therefore, it is relevant to note that behavioral studies have characterized the consequences of brain lesions restricted to hippocampus and ventral or dorsal striatum. Hippocampal lesions do impair spatial navigation performance in the WM test (Marston *et al.*, 1993), and this defect is believed to result from impaired formation of new relational memory engrams and from defects in spatial processing. A lesion of the nucleus accumbens, a region of the ventral striatum connected with the hippocampus and the prefrontal cortex, slightly impairs accuracy of WM navigation (Annett *et al.*, 1989) by disrupting the execution of complex movement patterns. In contrast, destruction of the dorsal striatum has no effect on spatial

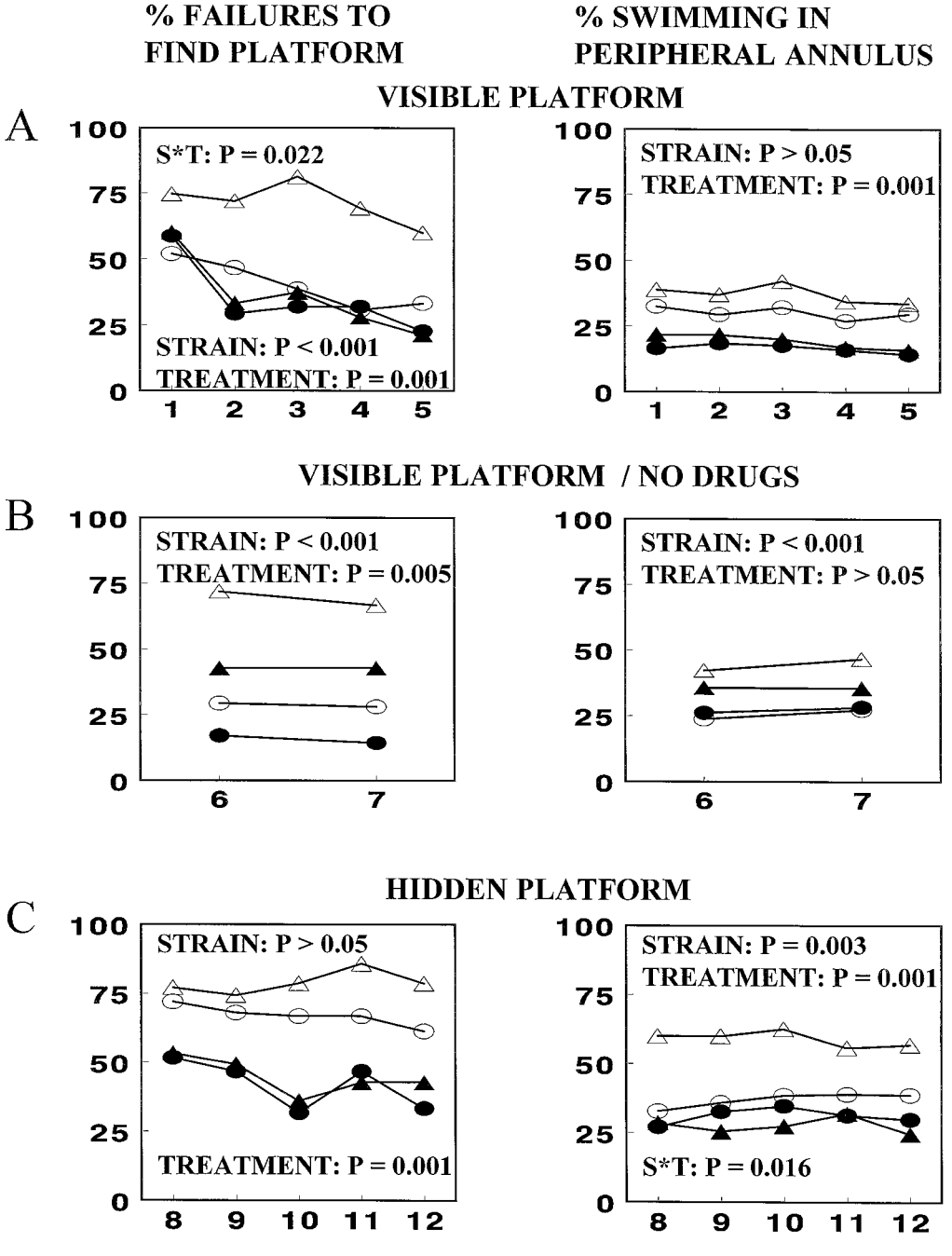

Fig. 2. Platform finding (percent failures to find platform) and the time spent in the peripheral annulus (percent swimming in peripheral annulus) of OE and WT mice treated with ATI 300 μg/kg. *Left*, percentage of mice that failed to find the platform (*y*-axis). *Right*, time spent in the peripheral incorrect annulus that did not contain the platform (*y*-axis). The *x*-axis shows the training day. Daily group means are shown. The *p* values of significant strain and treatment effects and strain × treatment (*S* × *T*) interactions are shown. ○, WT saline; △, OE saline; ●, WT ATI 300 μg/kg; ▲, OE ATI 300 μg/kg. A, Atipamezole (*ATI*; 300 μg/kg subcutaneous) stimulated OE mice WM visible platform finding more than WT mice and decreased swimming in the peripheral annulus to the same extent in both strains. B, During drug-free training days, the WT and OE mice previously treated with ATI 300 μg/kg found the visible platform more effectively than their saline controls. C, ATI 300 μg/kg improved hidden platform finding equally in OE and WT mice, but it decreased swimming in the peripheral annulus more effectively in OE mice.

navigation and selectively impairs cue navigation, indicating that this region is involved in a brain memory system that processes a different kind of information than the hippocampal system. Therefore, these previous studies suggest that α_{2C}-ARs may modulate those brain areas involved in nonspatial and spatial memory and also affect execution of learned escape behaviors. Indeed, we found that KO mice were less sensitive than CKO mice to the defect in spatial navigation induced by DEX 10 μg/kg. This finding with KO mice supports our contention that α_{2C}-overexpression in anatomically relevant brain areas is responsible for the performance defect in OE mice.

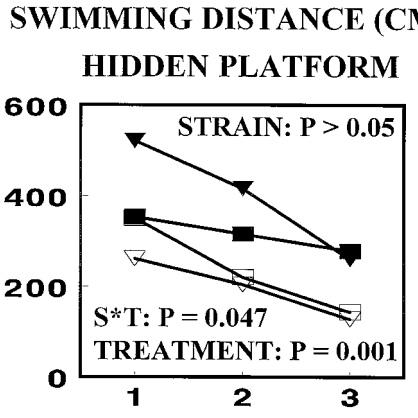

Fig. 3. Swimming distance of KO mice and their CKO treated with DEX 10 μg/kg (y-axis). The x-axis shows the training day. Daily group means are shown. The p values of significant strain and treatment effects and strain $\times$ treatment ($S \times T$) interactions are shown. ▽, CKO saline; □, KO saline; ▼, CKO DEX 10 μg/kg; ■, KO DEX 10 μg/kg. Saline-treated CKO and KO mice had equal swimming distances. KO mice were less sensitive to the DEX 10 μg/kg-induced increase in swimming distance than were CKO mice.

Our control studies indicated that the OE mice were not different in other behavioral and physiological measures, providing evidence for the specificity of the WM abnormality. We analyzed PA and OF behaviors and cortical electrical arousal, but no differences between the OE and WT mice were detected. The lack of a strain difference in the OF test indicates that differences in anxiety do not play a role in the abnormal WM search pattern of OE mice. The PA performance of OE mice was normal, suggesting that it is not a simple defect in stimulus-response learning, which is the foundation of the impaired WM accuracy of OE mice (Thomas, 1996). In addition, we tested the action of DEX to increase and ATI to suppress cortical slow waves and observed that α_{2C}-AR OE did not modulate the effects of α_2-AR active drugs on cortical EEG arousal (Riekkinen *et al.*, 1990, 1993). EEG measurements revealed no differences between OE and WT mice, thereby ruling out altered arousal state as the cause of the WM defect of OE mice. Also, the defects in performance after DEX during PA training and testing trials and those during the visible platform navigation stage were of equal magnitude in WT and OE mice. These defects in WT and OE mice are likely to result from the sedative effects of DEX. The need for a greater DEX dose to disrupt WM navigation than that needed to interfere with PA behavior may be simply related to the high arousal and pronounced LC firing rate occurring during the WM testing.

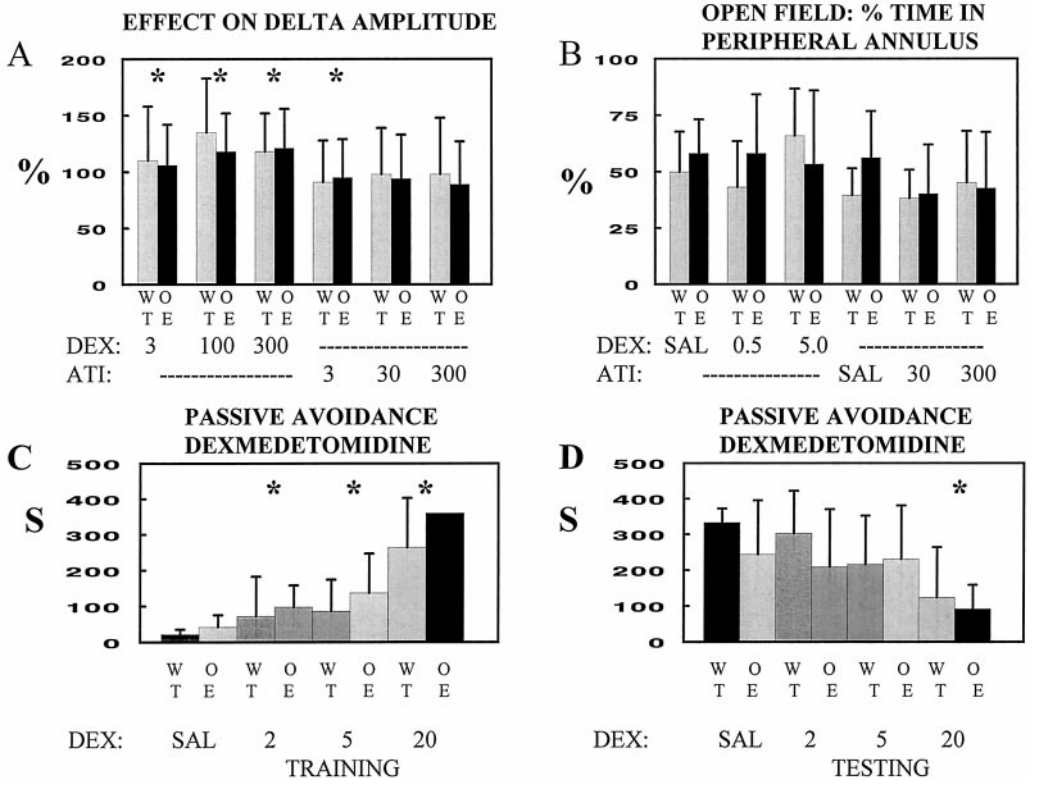

Fig. 4. A, DEX 3–300 μg/kg increased relative (vehicle = 100%) Δ (1–4 Hz) amplitude in WT and OE mice measured from frontal epidural EEG electrodes. ATI 3 μg/kg decreased Δ amplitude, but ATI 30–300 μg/kg had no effect. B, In an OF test, DEX 0.5–5 μg/kg and ATI 30–300 μg/kg had no effects on ambulation in the peripheral annulus in WT and OE mice. C, Passive avoidance training latencies of WT and OE mice did not differ. DEX 2–20 μg/kg increased training latency equally effectively in both strains. D, Passive avoidance testing latencies of WT and OE mice did not differ. DEX 2–5 μg/kg had no effect on testing latency, whereas DEX 20 μg/kg decreased training latency as effectively in both strains. *, $p < 0.05$ versus saline-treated control mice.

The decreased swim speed in OE mice may not be related to overexpression of α_{2C}-AR because a high dose of ATI (300 μg/kg) retarded swim speed in OE and WT mice to the same extent. Therefore, this difference between the WT and OE mice is the only one that may not be specifically related to overexpression of α_{2C}-AR.

In summary, our results revealed that OE mice were impaired only in the WM test, which requires organization of a complex escape behavior. We also observed an alteration in the dose-response relationship of the beneficial effect of an α_2 antagonist on WM performance in α_{2C}-AR OE mice. The poor WM performance of OE mice and the improving effect of ATI on navigation performance are difficult to attribute simply to disrupted learning and memory. It is likely that α_{2C}-AR overexpression may not impair learning *per se* because the OE and WT mice had parallel learning curves, ATI 300 μg/kg treatment did not affect the slope of the learning curves, and ATI did not improve memory consolidation. Therefore, α_{2C}-ARs may modulate frontostriatothalamic feedback loops (Scheel-Krüger and Willner, 1991; Coull *et al*., 1995) and influence complex spatial navigation behaviors. These results raise the possibility that antagonists selective for the α_{2C}-AR subtype and agonists devoid of any α_{2C}-AR affinity could modulate cognition more favorably than subtype-nonselective drugs. For example, α_{2C}-AR subtype-selective antagonists could be effective in the treatment of some of the cognitive dysfunctions that are characterized by impaired frontostriatothalamic functions, such as planning and controlling of complex escape behavior, and are susceptible to α_2-AR subtype-nonselective antagonists. Furthermore, α_2-AR agonists devoid of any α_{2C}-AR affinity may be also more effective than the currently available α_2-AR agonists in enhancing memory and impulse control functions of prefrontal cortex (Steere and Arnsten, 1994).

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Send reprint requests to: Dr. Paavo Riekkinen, Jr., Department of Neuroscience and Neurology, University of Kuopio, P.O.B. 1627, FIN-70211 Kuopio, Finland. E-mail: paavojr.riekkinen@uku.fi