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Dexmedetomidine Reduces Response Tendency, but Not Accuracy of Rats in Attention and Short-Term Memory Tasks

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RUOTSALAINEN, S., A. HAAPALINNA, P. J. RIEKKINEN SR AND J. SIRVIÖ. *Dexmedetomidine reduces response tendency, but not accuracy of rats in attention and short-term memory tasks.* PHARMACOL BIOCHEM BEHAV **56**(1) 31–40, 1997.—The present study investigated the role of α_2-adrenergic mechanisms in the performance of motor responses, attention and short-term memory in rats. A low dose (3.0 μg/kg, s.c.) of dexmedetomidine, an α_2-adrenoceptor agonist, reduced response tendency in an attentional task and a working memory task, but it did not affect the choice accuracy of rats. Atipamezole (300 μg/kg), an α_2-adrenoceptor antagonist, increased anticipatory responding. Although atipamezole did not affect the number of omissions, it reversed the effects of dexmedetomidine on that parameter. We also investigated the effects of dexmedetomidine in rats with partial destruction of noradrenergic nerves induced by the neurotoxin DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride). On its own, DSP-4 treatment did not affect choice accuracy or behavioural activity of rats in the attentional task. The effects of dexmedetomidine (0.3-3.0 μg/kg) on anticipatory responses did not differ between controls and DSP-4 group. Furthermore, the effect on omissions was not consistently diminished in DSP-4 treated rats. These results suggest that the activation of postsynaptic α_2-adrenoreceptors may be responsible for dexmedetomidine-induced reduction of response tendency while attention and short-term memory are not markedly affected. **Copyright © 1997 Elsevier Science Inc.**

α_2-adrenoceptors Atipamezole Attention Dexmedetomidine Noradrenergic system Rat

ANATOMICAL findings have shown that the noradrenaline-containing neurons of the locus coeruleus form one of the ascending systems of the brainstem innervating the forebrain (15). Noradrenaline is thought to regulate cortical desynchronization/synchronization (4,33), to increase the signal-to-noise ratio in the neocortex (30) and the responsivity of thalamocortical relay neurons (6,34) as well as to facilitate excitatory and inhibitory responses in the limbic system (36,40). Electrophysiological studies demonstrate that the noradrenergic system plays an important role in arousal, vigilance and responses to novel, salient stimuli (3,19). Psychopharmacological studies further suggest a role for the noradrenergic system in the processes underlying attention and learning (10,14,17,32).

The firing rate of the neurons in the locus coeruleus is regulated by α_2-adrenoceptors (1,2,9). The activation of these receptors causes the autoinhibition of noradrenergic neurons, whereas the blockade of these receptors increases the firing rate of locus coeruleus neurons and increases the release of noradrenaline in brain (24). The blockade of α_2-adrenoceptors can also increase the responsiveness of locus coeruleus neurons to excitatory stimulation (42).

Recent experiments in this laboratory have examined the role of α_2-adrenoceptors in mediating behavioural activity, vigilance and sustained attention by investigating the effects of selective α_2-adrenoceptor agents on the performance of adult rats in a 5-choice serial rection time task (22,43,44). This task, which can be considered to assess sustained attention, requires an animal to detect and respond to brief flashes of light in spatially diverse locations (12). Dexmedetomidine, an α_2-adrenoceptor agonist, dose-dependently increased the amount of omissions, latency of responses and decreased the number of premature responses, while it did not impair choice accuracy of rats in the standard version of this task (44).

The lowest dose (3 μg/kg) of dexmedetomidine that pro-

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duced a consistent reduction in response tendency of rats in the attentional task has been found to reduce slightly the levels of the metabolite of noradrenaline in the cerebrospinal fluid indicating a reduced release of this neuromodulator (27). Since heterosynaptic α_2-adrenoceptors also exist in the central nervous system, and their number may exceed those of autoreceptors in the brain (20), the aim was to study if the dexmedetomidine-induced reduction in the response tendency of rats is related to a decreased activity of locus coeruleus by investigating whether the destruction of the noradrenergic axons originating from the locus coeruleus can influence the efficacy of dexmedetomidine to reduce response tendency in rats. Another aim was to confirm the pharmacological specificity of the behavioural effects by studying whether atipamezole, an α_2-adrenoceptor antagonist, could reverse dexmedetomidine-induced sedation. Since the attentional task has also a short-term memory component (inter trial interval), the effects of dexmedetomidine on short-term memory task (delayed non-matching to position) performance was investigated in order to study in more detail the influence of this α_2-adrenoceptor agonist on cognition.

MATERIALS AND METHODS

Animals

In the present experiments, male Han:Wistar rats ($n = 20$) were used. The rats were 3 months old at the beginning of behavioral training. The rats were singly housed in stainless steel shoe box cages in a controlled environment (temperature 20°C , humidity 50-60 %, lights on 0700-2100). During training and testing, the rats were deprived of food for 16-18 hours before daily training or testing. After daily behavioral training or testing, the rats received about 12 grams of food pellets (Special Diet Service, England), so that they were maintained at approximately 85 % of free-feeding weight. Water was available ad libitum except in the test apparatus.

Drugs

Dexmedetomidine and atipamezole were produced by Orion Corporation, Farmos Pharmaceuticals, Turku, Finland.

Medetomidine (4(5)-[1-(2,3-dimethylphenyl)ethyl]-imidazole) is a potent, highly specific and selective α_2-adrenoceptor agonist. In studies with isolated organs and in receptor binding studies medetomidine has a higher intrinsic activity, higher affinity at α_2-adrenoceptors and higher relative α_2/α_1-selective ratio than other tested α_2-adrenoceptor agonists (detomidine, clonidine, UK 14,304 or xylazine). Medetomidine inhibits dose dependently release of noradrenaline, serotonin and dopamine in rat brain (26). Medetomidine does not have affinity or effects on any tested receptors other than α_2-adrenoceptors (47), and it has no selectivity for α_{2A}- or α_{2B}-adrenoceptor subtypes (46). Medetomidine is a racemic mixture of two enantiomers. It has been clearly shown that the pharmacological effects of medetomidine are caused by its dextro enantiomer, dexmedetomidine (27,37).

Atipamezole (4-(2-ethyl-2,3-dihydro-1H-inden-2-yl)-1H-imidazole) is a relatively novel, highly selective and specific α_2-adrenoceptor antagonist (38,48). In receptor binding studies, atipamezole is reported to have about 100 times higher affinity for α_2-adrenoceptors and over 100 times higher α_2/α_1 selectivity ratio than idazoxan and yohimbine. In studies with isolated organs, atipamezole is a more potent α_2-adrenoceptor antagonist and has about 200 times higher relative α_2/α_1 blocking ratio than idazoxan (48). Atipamezole has an almost equal affinity for the different α_2-adrenoceptor subtypes (31). Atipamezole penetrates rapidly into brain (5), and it causes a dose-dependent increase in the release of central noradrenaline and serotonin (38).

DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride) was purchased from Research Biochemicals Inc., USA. This toxin has been shown to destroy noradrenergic cell in the locus coeruleus as well as their axons (1,16).

Behavioural Training and Testing in 5-Choice Serial Reaction Time Task

Apparatus. The test apparatus (7), which was made in the Technical Center (University of Kuopio, Finland) consisted of a 25 × 25 cm aluminium chamber with a curved rear wall. Set in the curved wall were 9, 2.5 cm square holes, 4 cm deep and 2.5 cm above floor level. Each hole had an infra-red photocell beam crossing the entrance vertically and illuminating a photo-electric cell. A standard 3 W bulb at the rear of the hole provided illumination for that hole. The entrances to holes 2, 4, 6 and 8 were blocked with a metal cap. Food pellets (45 mg, dustless, Bioserv. Inc., New Jersey, U.S.A.) could be dispensed automatically into a magazine at the front of the chamber. Access was gained to the magazine through a Perspex door (=panel). The distances from the panel to the illuminated holes at the rear of the box were all 25 cm. The chamber was illuminated by a 3-W house-lamp mounted in the roof. The animals were introduced to the chamber through

TABLE 1

THE EFFECT OF DSP-4 TREATMENT (2 × 50 MG/KG, I.P.) ON THE LEVELS OF NORADRENALINE (NA), SEROTONIN (5-HT), 5-HYDROXYINDOLE ACETIC ACID (5-HIAA), DOPAMINE (DA), HOMOVANILLIC ACID (HVA), 3,4-DIHYDROXYPHENYLACETIC ACID (DOPAC) IN DIFFERENT AREAS OF THE BRAIN

		Controls ($n = 6$)	DSP-4 ($n = 5$)
NA			
	Frontal cortex	143.3 ± 23.9	15.3 ± 8.5**
	Hippocampus	259.9 ± 54.3	26.1 ± 12.5**
	Striatum	131.2 ± 52.3	65.2 ± 18.1*
	Hypothalamus	445.5 ± 196.1	373.6 ± 121.9
5-HT			
	Frontal cortex	127.8 ± 95.9	78.7 ± 41.5
	Hippocampus	167.1 ± 69.6	101.5 ± 29.1
	Hypothalamus	159.7 ± 43.7	225.4 ± 61.8
5-HIAA			
	Frontal cortex	275.0 ± 34.0	192.9 ± 60.6
	Hippocampus	524.8 ± 237.7	252.5 ± 44.3**
	Striatum	457.5 ± 91.0	468.6 ± 55.3
	Hypothalamus	510.6 ± 135.0	625.3 ± 152.2
DA			
	Striatum	4297 ± 1034	5535 ± 1362
DOPAC			
	Striatum	2509 ± 932	2249 ± 989
HVA			
	Striatum	361.8 ± 87.0	368.6 ± 76.7

Results are expressed as mean ± S.D. Note, 5-HT levels were not assayed in the striatum. The levels of dopamine and its metabolites in the cortex and hippocampus were not clearly above the detection limit.

* $p < 0.05$; ** $p < 0.01$ using Mann-Whitney *U*-test.

a Perspex door in the top half of the front wall. The apparatus was housed in a dark, soundproof compartment. On-line control of the apparatus and data collection were performed using microprocessors which were programmed using Spider (Paul Fray Ltd., Cambridge, U.K.). The stimulus intensity reductions were achieved by adding resistors in series with the light bulbs.

Training

The rats (= 13) were trained in the following manner to discriminate spatially a brief visual stimulus, presented randomly by the computer in one of the five holes (from left, holes 1, 3, 5, 7 and 9). On the first three days of behavioural testing, all of the rats were magazine-trained by being placed in the chambers for 15 min with the house-light on and the food tray containing 30 - 40 food pellets. On the next day, the rats were placed in the chambers for 15 minutes and a food pellet was delivered every 10 s into the magazine. The house-light was on during this phase. In the third phase, one of the holes was illuminated all the time during the 15 minute training period and every time the rat made a response (nose-poke) on the illuminated hole it was reinforced by a food pellet on the magazine.

After learning this, the rats entered the next phase, which started by the free delivery of a single food pellet. The first trial started when the panel was opened to collect the food pellet. After a fixed delay (intertrial interval), the light at the rear of one of the holes was illuminated for a short period (stimulus duration). The light stimulus was presented in each of the holes for an equal number of times during each complete session, and the order of presentations was randomized by the computer. Responses (nose-pokes) by the rat in the illuminated hole and responses in that particular hole for a short period after the illumination (the limited hold) were rewarded with the delivery of a food pellet and a correct response was recorded. The next trial was initiated when a rat opened the panel to collect a food pellet. A response in any other hole (incorrect response) or a failure to respond at all (omissions) resulted in the termination of a trial which was signalled by turning the roof light off for 4 seconds (time-out). Therefore, if the rat was facing in the wrong direction when the visual stimulus was presented on a hole it would not detect it and this trial resulted in an omission and a period of time-out. Any response made during the time-out period restarted the time-out. Responses made in the holes during the intertrial interval period were recorded as premature (or anticipatory) responses and responses made in the panel during the intertrial interval period were recorded as perseverative responses. Intertrial interval hole responses resulted in a period of time out. The next trial was initiated when a rat opened the panel after the completion of a time-out period. The latency between the onset of the stimulus and response, whether correct or incorrect, was measured, as well as the latency to collect the earned food pellet following the completion of a correct re-

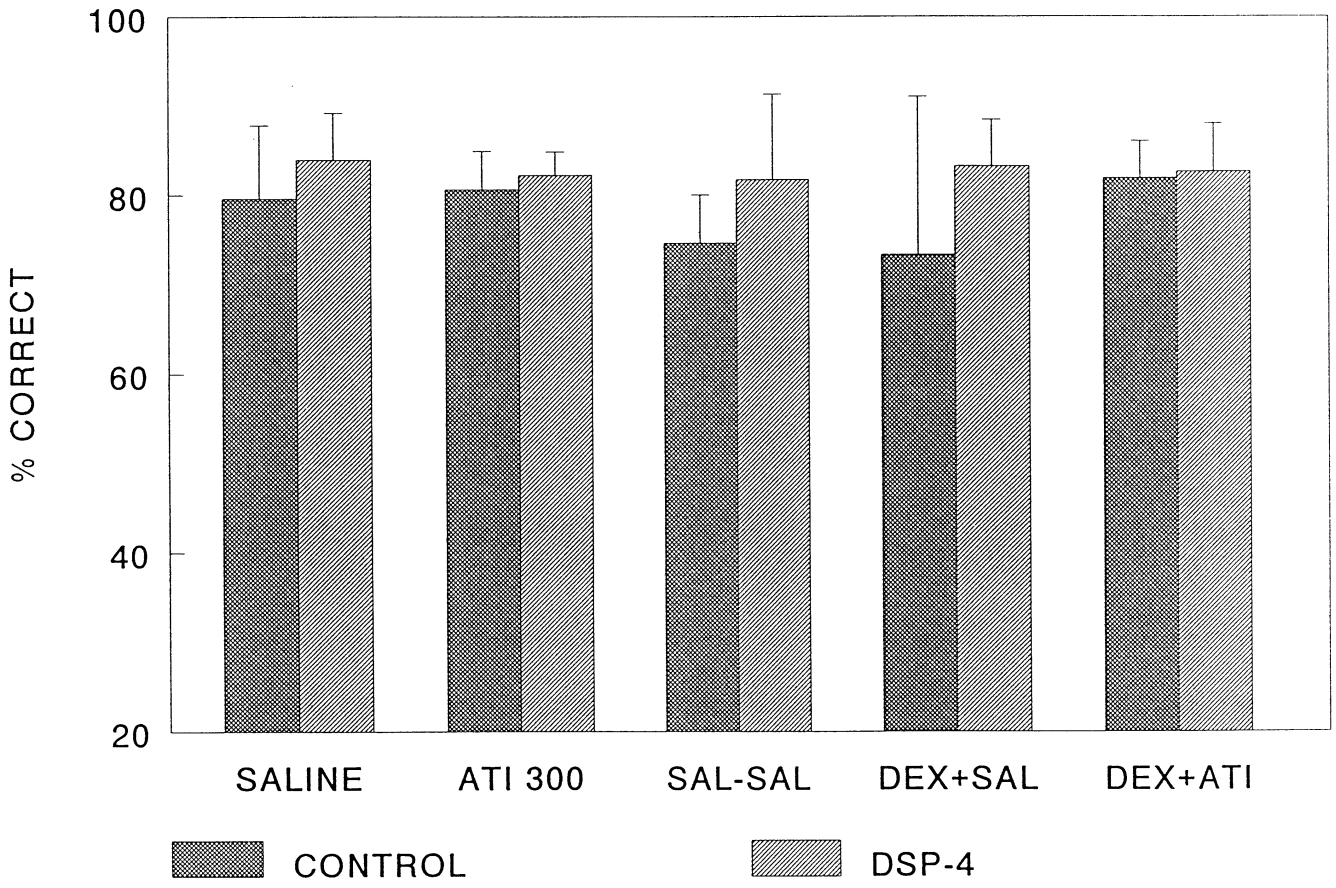

FIG. 1. The effects of atipamezole 300 μg/kg (ATI) and dexmedetomidine 3 μg/kg (DEX) on the percent correct responses in control and DSP-4 treated rats. Results are expressed as mean + S.D.

sponse. Each daily training session (three sessions a week) consisted of 20 min of training. During the first session of training, the stimulus duration and limited hold periods were set at 4.0 s and 0.5 s, respectively. These durations were then progressively altered to 0.5s and 3.5 s, respectively during the training. The intertrial interval and time out were both set at 5.0 s and 4.0 s, respectively.

Each rat was trained on this schedule until a stable performance level was attained. It took about 25 training sessions to reach a stable level when no further improvement in performance could be observed. Baseline data were collected from the last three training sessions at the normal parametric conditions (Table 1). Thereafter, the rats were also tested at the shortened stimulus duration (25 cs) and reduced stimulus intensity (50% of normal).

Behavioural Variables. The following parameters are analyzed in each session: 1) trials = the total number of trials (correct + incorrect) made during a 30 min testing session; 2) intertrial interval responses = the number of premature responses on the holes or perseverative responses in the panel made during the intertrial interval; 3) omissions = the number of errors of omissions made, ie. the number of times when the rats failed to respond at all to the visual stimulus; 4) response latencies = the latency to respond (the time between the onset of the stimulus and a nose-poke) was recorded separately for correct and incorrect responses; 5) magazine latency = the latency to collect earned food pellets from the magazine after a correct response; 6) discriminative accuracy = the proportion of correct responses (correct responses/correct + incorrect responses) made, expressed as a percentage.

Noradrenergic Lesions With DSP-4 and Drug Testing

The rats were changed to a free feeding schedule after the acquisition of the task. Two weeks later the rats were treated with noradrenergic neurotoxin (= 7) or vehicle (= 6). DSP-4 was dissolved in saline, and it was administered intraperitoneally (50 mg/kg) twice. The injections (4 ml/kg) were separated by 24 hours. One DSP-4 treated rat died after the first injection.

After the recovery period (one week), the rats were food deprived as described above. One week later, the testings of the rats were reinstated. Each session lasted for 30 minutes. The rats were tested every second or third day for five sessions. The last two sessions were collected for the analysis of behavioural data after the treatment (Table 1). Then, the rats were habituated to the subcutaneous injections.

After post-lesioning habituation, the testings of α_2 agents were started. At that time, the rats were 8-month-old. Dexmedetomidine hydrochloride and atipamezole hydrochloride were dissolved in sterile saline. Drug solutions were injected subcutaneously (s.c.) (0.5 ml/kg) 30 minutes before testing sessions. First, the rats were treated with saline, dexmedetomidine 0.3, 1.0 or 3.0 μg/kg in a counter balanced order before testings which were done every second day. Then, all the rats were tested once without any injections.

Next, the rats were treated with saline or dexmedetomidine 1.5 μg/kg in a counter balanced order and tested at the baseline conditions. Then, all the rats were treated with saline, and tested at the curtailed stimulus duration (25 cs). After this habituation, dexmedetomdine 1.5 μg/kg and saline were injected in a counter balanced order, and the rats were tested using 25 cs for stimulus duration. Then, all the rats were treated with saline, and they were tested at the reduced stimulus intensity (50% of the normal). After this habituation, the rats were treated with saline or dexmedetomidine 1.5 μg/kg in a counter balanced order before testings at the reduced stimulus intensity. Trials were performed every second day. The data of this part is not shown.

Two weeks later, the rats were treated with saline and atipamezole (300 μg/kg) in a counter balanced order, and then saline-saline, dexmedetomidine 3.0 μg/kg-saline, dexmedetomidine-atipamezole 300 μg/kg in a counter balanced order. Testing was performed every second day. During the washout periods, the rats were occasionally tested without injections. They performed normally in those situations.

After the experiment (4 months after DSP-4/vehicle treatment), the rats were decapitated. The brains were removed from the skull and stored at −75°C.

Biochemical Analysis. Before the neurochemical analysis, the brain was thawed and the cerebral cortex, hippocampus,

TABLE 2

THE NUMBER OF TRIALS COMPLETED (TRIALS), THE PERCENT CORRECT RESPONSES (% CORRECT), INTERTRIAL INTERVAL RESPONSES (% ITI HOLE), OMISSIONS (% OMISSIONS), THE LATENCY OF CORRECT RESPONSES (CLATE, S) AND MAGAZINE LATENCY (MLATE, S) OF CONTROL AND DSP-4 TREATED RATS BEFORE (BASAL) AND AFTER THE INJECTIONS OF VEHICLE OR TOXIN (TRNT)

	Controls (n = 6)		Dsp-4 (n = 5)	
	Basal	Trnt	Basal	Trnt
Trials	148 ± 7	179 ± 44	168 ± 31	173 ± 46
% Correct	70.5 ± 6.8	72.4 ± 8.7	80.0 ± 8.1	79.7 ± 7.5
% Iti hole	30.6 ± 11.8	29.8 ± 11.1	28.8 ± 7.4	23.2 ± 7.8
% Omissions	16.0 ± 8.4	10.6 ± 7.1	12.8 ± 5.8	12.5 ± 5.2
Clate	0.88 ± 0.09	0.87 ± 0.10	0.85 ± 0.08	0.85 ± 0.09
Mlate	1.63 ± 0.63	1.62 ± 0.56	2.26 ± 0.57	2.48 ± 2.32

Results are expressed as mean ± S.D.

Paired-wise testing (Basal vs. Trnt) did not reveal significant differences (paired t-test, $p > 0.01$) in any parameter between control group or DSP-4 group. Between group testing did not reveal significant differences (t-test, $p > 0.1$) in any parameter either before (Basal) or after treatments (Trnt).

TABLE 3

THE EFFECTS OF DEXMEDETOMIDINE (0.3 − 3.0 μG/KG) ON THE PERCENT CORRECT RESPONSES, INTERTRIAL INTERVAL RESPONSES AND OMISSIONS IN CONTROLS AND DSP-4 TREATED RATS

		Saline	Dex 0.3	Dex 1.0	Dex 3.0
% Correct	Control	78.6 ± 8.2	74.6 ± 9.8	77.8 ± 7.8	79.7 ± 7.5
	Dsp-4	84.3 ± 7.5	84.2 ± 7.0	86.3 ± 4.9	85.6 ± 6.8
% Iti hole	Control	20.2 ± 18.2	20.4 ± 13.0	12.8 ± 7.8	8.1 ± 11.4*
	Dsp-4	20.5 ± 9.2	22.8 ± 8.7	23.9 ± 10.7	10.8 ± 10.0*
% Omissions	Control	16.4 ± 8.4	15.2 ± 5.7	15.6 ± 9.1	40.1 ± 13.7*
	Dsp-4	9.1 ± 4.9	9.0 ± 7.4	13.2 ± 7.1	20.0 ± 8.0**

Results are expressed as mean ± S.D.
* $p < 0.05$; ** $p < 0.01$ as compared to saline using paired *t*-test.

striatum and hypothalamus were dissected. Brain tissue was homogenized and prepared for the analysis of monoamines and their metabolites as described previously (23). Noradrenaline, dopamine and 3,4-dihydroxyphenylacetic acid as well as serotonin, 5-hydroxyindoleacetic acid and homovanillic acid were measured using high performance liquid chromatography with electrochemical detection as described previously (23).

Statistical Analysis. Paired -test (baseline performance vs. performance after treatment (saline or DSP-4)) and between group -test were used to analyze the effects of DSP-4 treatment on the performance of rats at normal condition without any injections. The differences between groups in the neurochemical parameters were tested using Mann-Whitney U-test.

Multivariate analysis of variance (MANOVA) was used to analyze the treatment effect (saline and different doses of a drug) and group effect as well as interactions between these effects in the percent correct responses, the number of trials and omissions, the latency of correct and incorrect responses as well as food collection. Before MANOVA analysis, the percent correct, ITI hole responses and omissions data were

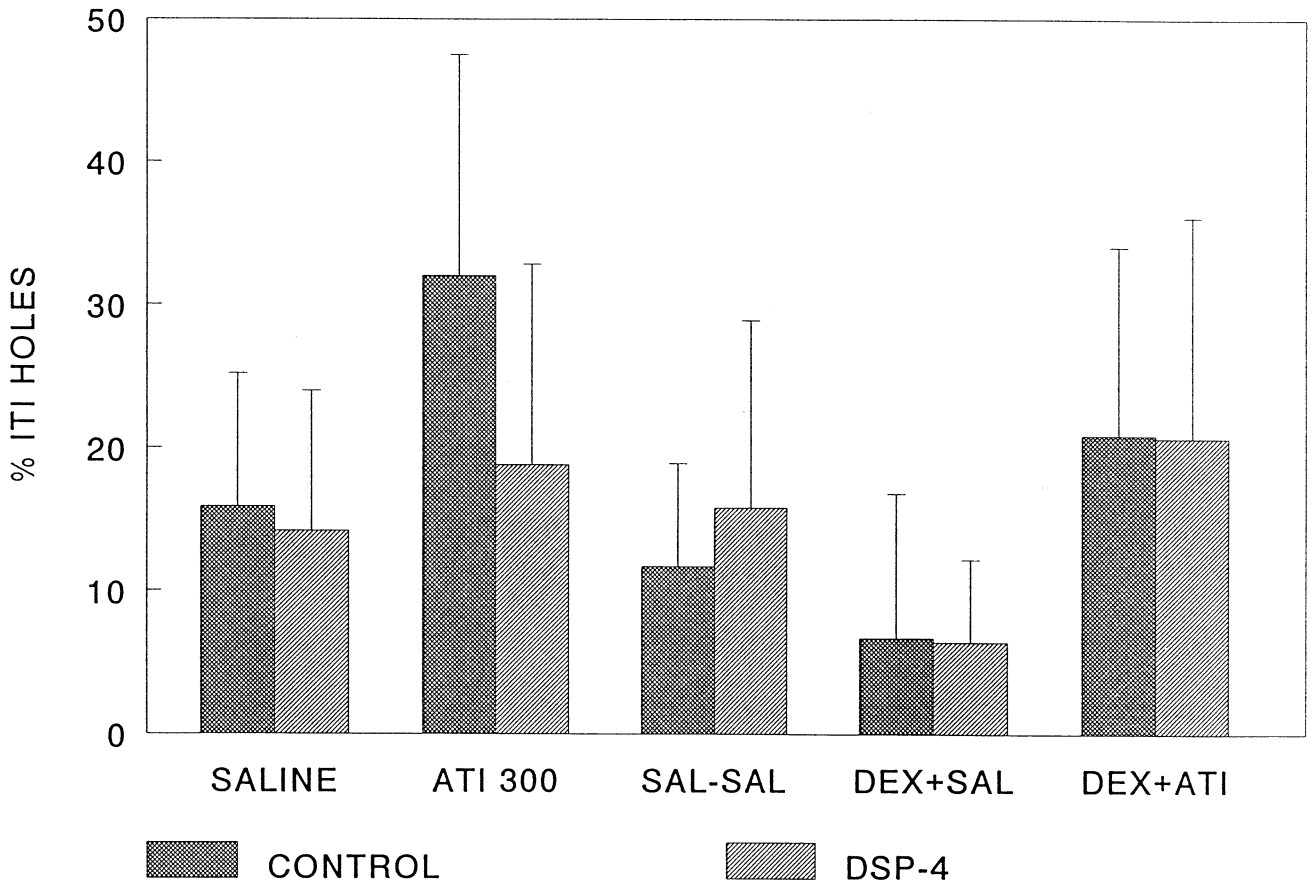

FIG. 2. The effects of atipamezole 300 μg/kg (ATI) and dexmedetomidine 3 μg/kg (DEX) on the probability of hole responses during intertrial interval in control and DSP-4 treated rats. Results are expressed as mean + S.D.

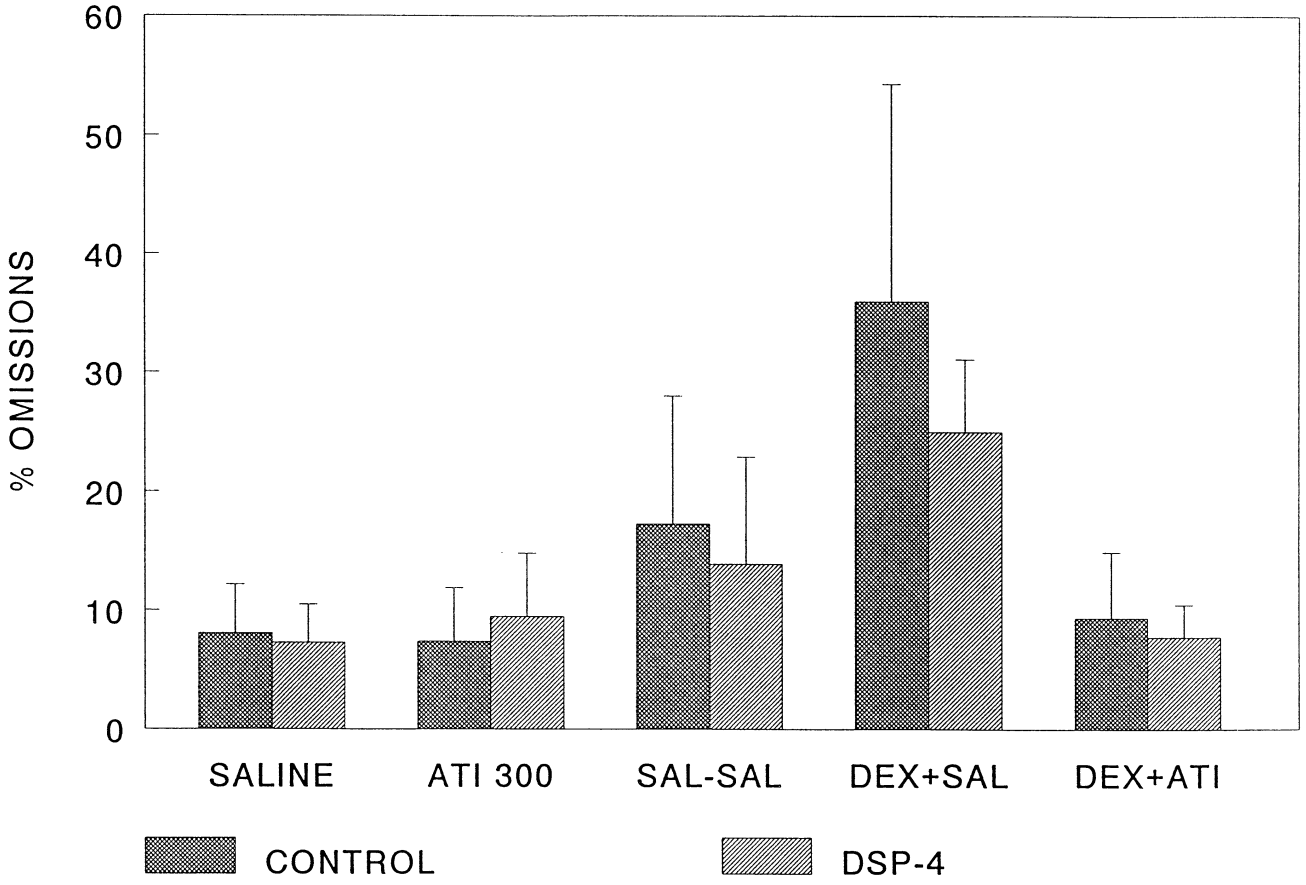

FIG. 3. The effects of atipamezole 300 μg/kg (ATI) and dexmedetomidine 3 μg/kg (DEX) on the probability of omissions in control and DSP-4 treated rats. Results are expressed as mean + S.D.

transformed using arcsine transformation. The number of trials and latency data were transformed using square root and logarithmic transformations, respectively. If MANOVA revealed an overall treatment effect, a post-hoc two-tailed *t*-test was used to analyze differences between treatments (saline versus different doses of a drug).

Behavioral Training and Testing in the Delayed Non-Matching to Position Task

Apparatus. Testing was conducted in two operant chambers equipped with two retractable levers and a food dispenser (Campden Instruments, London, UK). The operant chambers were under the online control of microprocessors (Paul Fray Ltd, Cambridge, UK). The food dispenser delivered 45 mg pellets (Campden Instruments, UK).

Training. Rats (n = 7) were habituated to the chambers with two retractable levers retracted and trained to collect food pellets and to associate the click of the dispenser plus illumination of the panel light with pellet delivery. During this training (phase 1), a pellet was delivered every time the rat made a nose poke into an illuminated pellet magazine. If the rat did not react within 20 s the illumination of the magazine was turned off for 5 s. The rats were trained 15 min/day until they learned to obtain at least two pellets/min. In the next phase, the rats learned to associate the pressing of a lever with the delivery of a food pellet. Both levers were inserted and every time when the rat pressed a lever, a food pellet was delivered into the magazine, which was illuminated. If the rat did not respond within 20 s, the levers were retracted for 5s. In phase 3, rats learned to press a lever (either right or left) when it was inserted into a chamber in order to get a food pellet. The right or left lever was inserted randomly and, if the rat pressed the lever, a food pellet was delivered and magazine was illuminated. Then, the lever was retracted, and after 5-s period, one of the levers was inserted once again. If the rat did not press the lever within 20 s, the lever was retracted and the house light was turned off for 5 s. In the next phase, rats were trained for non-matching to position task (without a delay). A right or left lever (sample) which was selected randomly was inserted into the operant chamber. When the rat pressed the sample lever, it was retracted and a magazine was illuminated, but no food pellet was delivered at that time. When a rat made a nose poke into a magazine, a magazine light was turned off and both levers were inserted. In this choice phase, the pressing of the non-sample lever was reinforced with a delivery of a food pellet into an illuminated magazine. If the rat pressed the sample lever once again, no food pellet was delivered and the house light was turned off for 5 s. After a 5-s period, a new sample lever was inserted. If the rat did not press a sample lever or one of the choice levers within 20 s, the house light was turned off for 5 s and a new sample lever was inserted after a 5-s interval.

After rats were trained for 10 days in the non-matching to position phase, delays (0,1,2,4,8,16 s) before inserting the choice levers were included (i.e. delayed non-matching to posi-

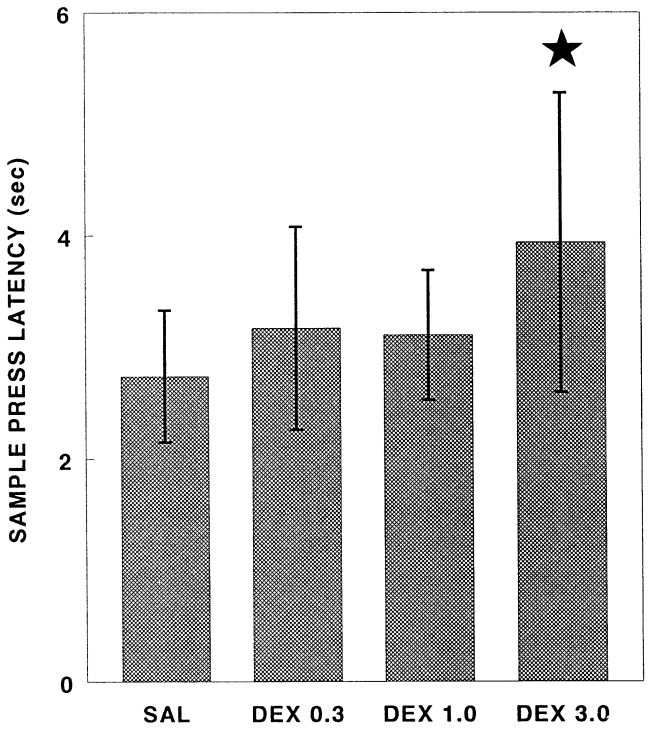

FIG. 4. The effects of dexmedetomidine (0.3–3.0 μg/kg) on the sample press latency (mean ± S.D. in seconds) in the delayed non-matching to position task. * $p < 0.05$ as compared to saline treatment.

tion). During this testing the pressing of the sample lever started a delay, but both levers (choice phase) were not inserted until the delay had expired and the rat had made a nose poke into a magazine. (This is considered to reduce the likelihood that the rat uses the strategy of remaining waiting near to the correct choice lever). This phase was continued for 10 training days. After this training, longer delays (0,2,4,8,16,30) were introduced to the rats, and this phase was continued for 20 training days.

In this task, the number of trials completed, percent correct responses and latencies for responses were analysed. The percent correct responses at each delay (i.e. the forgetting curve) was a measure of the working memory. In control rats, it was 90-100% at 0-s delay, and it declined to near a chance level 50-60% with a 30-s delay.

Testing. The rats were habituated to the subcutaneous injections. Then, the rats ($n = 7$) were treated with saline, dexmedetomidine 0.3, 1.0 or 3.0 μg/kg (s.c.) in a counter balanced order 30 minutes before testings which were done every second day.

Statistical analysis of data. Behavioural data was analyzed using MANOVA which was used to analyze the treatment effects (saline and different doses of drugs) and the interaction of the treatment effect with delay. Before MANOVA analysis, data was normalized using appropriate transformations. Post-hoc tests e.g. 2-tailed *t*-test were used to compare different doses of a drug to a vehicle treatment.

RESULTS

5-Choice Serial Reaction Time Task

DSP-4 treatment significantly reduced the levels of noradrenaline in the cerebral cortex and hippocampus in five of six treated rats (Table 1). One DSP-4 treated rat which was excluded had noradrenaline levels 83% and 105% of control values in the cortex and hippocampus, respectively. Noradrenaline levels of DSP-4 treated rats ($n = 5$) were slightly reduced in the striatum, but not in the hypothalamus (Table 1). The levels of serotonin and its metabolite, 5-hydroxyindoleacetic acid, were slightly reduced in the same rats and brain areas which showed a marked reduction of noradrenaline. The levels of dopamine and its metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid) were not affected in the striatum (Table 1).

For the statistical analysis of behavioural data, the one DSP-4 treated rat without any marked noradrenaline depletion was excluded. The performance of the other DSP-4 treated rats in the 5-choice serial reaction time task did not significantly differ between pre-treatment and post-treatment period (Table 2).

Dexmedetomidine (0.3–3.0 μg/kg) did not affect the percent correct responses in the 5-choice serial reaction time task when assessed in a normal version of the task, since treatment effect ($F = 0.7$, df=3), group effect ($F = 4.3$, df=1) and their interaction ($F = 0.3$, df=15) did not reach significance ($p > 0.05$) (Table 3) In the analysis of hole responses during ITI, a significant overall treatment effect was found ($F = 6.5$, df=3, $p < 0.01$), and this effect did not differ between DSP-4 group and their controls. Post-hoc analysis revealed that dexmedetomidine 3.0 μg/kg decreased the probability of hole responses during intertrial interval in this task (Table 3). Another series of tests also indicated that even the lower dose of dexmedetomidine (1.5 μg/kg) slightly reduced ITI hole responses, and its effect did not differ between controls and DSP-4 treated rats (data not shown). In the analysis of omissions, a significant overall treatment effect was found ($F = 12.1$,df=3, $p < 0.01$), and this effect did not differ between controls and DSP-4 treated rats ($F = 2.6$, $p > 0.05$ for their interaction). The highest dose of dexmedetomidine, 3.0 μg/kg, increased the probability of omissions (Table 3).

When administered alone (ATI 300 vs SALINE), atipamezole (300 μg/kg) did not affect the percent correct responses (non-significant treatment effect $F = 0.2$, df=1, $p > 0.1$ and interaction between treatment and group $F = 0.5$, df=1, $p > 0.1$) (Fig. 1), while it increased the probability of hole responses during intertrial interval (a significant treatment effect $F = 10.5$, df=1, $p = 0.01$) which did not differ between controls and DSP-4 group (for interaction $F = 3.4$, df=1, $p > 0.05$) (Fig. 2), but it did not affect the number of omissions (a non-significant treatment effect $F = 0.7$, df=1, $p > 0.1$ and a non-significant interaction between atipamezole treatment and group $F = 2.7$, df=1, $p > 0.1$) (Fig. 3). Another testing (DEX+SAL vs SAL-SAL) replicated the effects of dexmedetomidine 3 μg/kg on the probability of ITI hole responses (a significant treatment effect $F = 8.8$, df =1, $p < 0.05$ and a non-significant interaction between dex treatment and group effect $F = 1.0$, df =1, $p > 0.1$) (Fig. 2) and omissions (a significant treatment effect $F = 8.8$, df=1, $p < 0.05$ which did not differ between controls and DSP-4 group (a non-significant interaction $F = 1.0$, df=1, $p > 0.1$) (Fig. 3), while it did not affect choice accuracy (a non-significant treatment effect $F = 0.0$., df=1, $p > 0.1$ and an interaction between treatment and group $F = 0.0$, df=1, $p > 0.1$) (Fig. 1). Importantly, though atipamezole did not affect the number of omissions when administered alone, it did block the dexmedetomidine (3 μg/kg)-induced increase in this parameter (DEX+SAL vs. DEX+ATI, $F = 19.1$, df=1, $p < 0.01$) (Fig. 3).

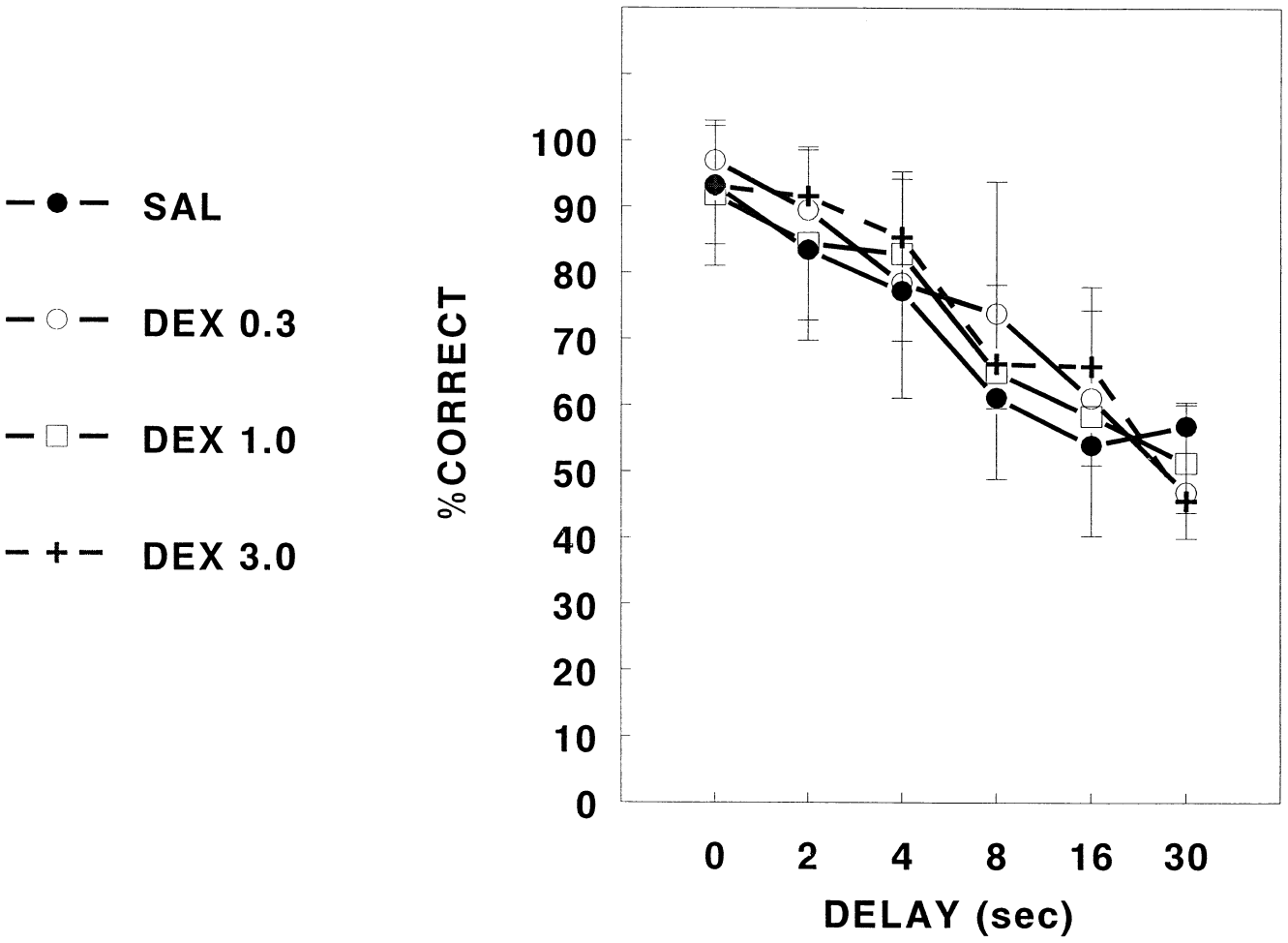

FIG. 5. The percent correct responses (the means of groups ± S.D. at each delay from 0 to 30 seconds) in the delayed non-matching to position task.

Delayed Non-Matching to Position Task

MANOVA revealed a significant treatment effect in the sample press latency [$F(3, 18)=4.5$, $p < 0.05$). Dexmedetomidine 3 μg/kg increased sample press latency as compared to saline treatment (2-tailed $p < 0.05$) (Fig. 4). Dexmedetomidine (0.3 - 3.0 μg/kg) did not affect spatial short-term memory, since MANOVA did not reveal a significant treatment effect in the percent correct responses across the delays [$F(3, 123)=1.2$, $p > 0.1$).

DISCUSSION

The pattern of DSP-4 induced reduction of noradrenaline in the brain is in agreement with previous studies (16,18,21,25). Furthermore, DSP-4 decreases the levels of noradrenaline in the cerebellum and spinal cord to the same extent as in the cerebral cortex and hippocampus (18). In the present study, two administrations of DSP-4 toxin were used to try to elicit a profound depletion of noradrenaline. This could explain the non-specific effects of the toxin on serotonin neurons. However, even a single dose of DSP-4 reduced serotonin levels by 20% one week after the administration of the toxin (21).

Previously, Carli et al. (7) found that a lesion of the dorsal noradrenergic bundle which markedly decreased the noradrenaline content of the forebrain did not affect the performance (choice accuracy, omissions, response speed or tendency) of rats in the standard version of a 5-choice serial reaction time task. In addition, a partial reduction of serotonin in the brain caused by p-chloroamphetamine did not impair the choice accuracy of rats in this task while it slightly enhanced their impulsivity in responding (Ruotsalainen et al., unpublished finding). The present results indicate that even the concurrent, marked decrease of noradrenaline and a slight decrease of serotonin in the cortex and hippocampus does not affect the choice accuracy under standard conditions.

The present data showed that dexmedetomidine dose-dependently reduced the probability of premature responses and increased the number of omissions indicating reduced response tendency in rats. In addition, it increased the latency to press the sample lever in the short-term memory task. Also, in line with the previous study (44), atipamezole increased the probability of premature responses in a reaction time task. This indicates that this treatment enhanced impulsivity in rats. Although atipamezole did not affect the number of omissions, it reversed the effects of dexmedetomidine on this parameter, which supports the idea that these agents acted via α_2-adreno-

ceptors. The present results also indicated that the choice accuracy of dexmedetomidine treated rats was preserved in the standard conditions of this attention task as well as in the short-term memory task.

It is clear that the lesioning of noradrenergic nerves and the administration of α_2-adrenoceptor agonist which depresses the activity of locus coeruleus neurons do not produce the same pattern of behavioral effects in the attention task. In addition, the comparison of the effects of dexmedetomine in DSP-4 group and their control group imply that DSP-4 did not influence the tendency of dexmedetomidine treated rats to respond during the intertrial interval, nor did DSP-4 treatment block the effect of dexmedetomidine (3 μg/kg) to increase the probability of omissions. In line with the present findings, Maze and his collegues have reported that sedative doses (>30 μg/kg) of dexmedetomidine were also effective in monoamine depleted rats (40). However, the limitations of lesion studies (incomplete depletion of noradrenaline and unknown site of action of a drug) to investigate α_2-adrenoceptor agonist-induced sedation has been discussed by Heal (20) using clonidine-induced sedation as an example. Therefore, it is important to note that Maze and his colleagues found that hypnotic responses to dexmedetomidine were seen also after injection of the drug into the locus coeruleus (13).

Since the present data indicate that α_2-adrenoceptors contribute to the modulation of impulsivity in responding, some possible mechanisms are discussed. The effects of α_2-adrenoceptor agents on behavioural activation could be, at least partly, mediated via the dopaminergic system. Pharmacological data on dexmedetomidine and atipamezole indicate that this α_2-adrenoceptor agonist can depress and the α_2-adrenoceptor antagonist can stimulate dopaminergic neurons (38). Interestingly, haloperidol, a dopamine receptor antagonist, increased the number of omissions and the latency to respond correctly (8), whereas amphetamine, a dopamine receptor agonist, increased impulsivity (the number of premature responses) and facilitated the speed of responding (12) in a 5-choice serial reaction time task.

An electrophysiological study has shown that dexmedetomidine dose-dependently increased the amount of high voltage spindle (spike and slow wave) activity in the cortical electroencephalogram of rats (34). A hypothesis has been proposed that this reduced thalamo-cortical arousal is due to activation of postsynaptic α_2-adrenoceptors which hyperpolarize thalamo-cortical neurons and increase the likelihood of their oscillations (6). However, Riekkinen Jr et al. (34) could not exclude the possibility that the systemic administration of an α_2-adrenoceptor agonist reduced the activity of cholinergic neurons in the brain stem, these neurons being important in the regulation of thalamo-cortical arousal. It has been suggested that the spindle-like oscillation does not permit an accurate signal transmission in the thalamo-cortical neurons (28). In the context of the present experiments, the oscillation promoting effect of dexmedetomidine could contribute to an increased probability of omissions in the vigilance task.

It is also important to consider the contributions of peripheral effects of adrenergic drugs and their interactions with the central mechanisms (29,39,45). Since DSP-4 has only modest and reversible effects on peripheral adrenergic nerves (21), these sites of action could remain intact to a greater extent even in the DSP-4 treated group.

In conclusion, our results suggest that systemic, acute administration of low doses of an α_2-adrenoceptor agonist does not markedly affect choice accuracy of rats under standard conditions, though it does reduce the likelihood to responding before and after the appearence of the stimulus. Furthermore, the effects of an α_2-adrenoceptor agonist on response tendency including reduction in impulsivity were not attenuated by a neurotoxin which is known to destroy noradrenergic axons originating from the locus coeruleus.

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REFERENCES

1. Aghajanian, G. K.; Cedarbaum, J. M.; Wang, R. Y. Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. Brain Res. 136:570–577; 1977.
2. Aghajanian, G. K.; VanderMaelen, C. P. Alpha 2-adrenoceptor mediated hyperpolarization of locus coeruleus neurons: Intracellular studies in vivo. Science 215:1394–1396; 1982.
3. Aston-Jones, G.; Shipley, M. T.; Ennis, M.; Williams, J. T.; Pieribone, V. A. Restricted afferent control of locus coeruleus neurones revealed by anatomical, physiological, and pharmacological studies In: Heal, D. J.; Marsden, C. A., eds. The Pharmacology of noradrenaline in the central nervous system. Oxford: Oxford University Press; 1990:187–247.
4. Berridge, C. W.; Foote, S. L. Effects of locus coeruleus activation on electroencephalographic activity in neocortex and hippocampus. J. Neurosci. 11:3135–3145; 1991.
5. Biegon, A.; Mathis, C. A.; Budinger, T. F. Quantitative in vitro and ex vivo autoradiography of the α_2 adrenoceptor antagonist (3H) atipamezole, Eur. J. Pharmacol. 224:27–38; 1992.
6. Buzsaki, G.; Kennedy, B.; Solt, V. B.; Ziegler, M. Noradrenergic control of thalamic oscillation: The role of α_2 receptors, Eur. J. Neurosci. 3:222–229; 1990.
7. Carli, M.; Robbins, T. W.; Evenden, J. L.; Everitt, B. J. Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats: Implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behav. Brain Res. 9:361–380; 1983.
8. Carli, M.; Samanin, R. Serotonin2 receptor agonists and serotonergic anorectic drugs affect rats' performance differently in a five-choice serial reaction time task. Psychopharmacology 106:228–234; 1992.
9. Cederbaum, J. M.; Aghajanian, G. K. Noradrenergic neurons of the locus coeruleus: inhibition by epinephrine and activation by the alpha-antagonist piperoxane. Brain Res. 112:413–419; 1976.
10. Clark, C. R.; Geffen, G. M.; Geffen, L. B. Catecholamines and Attention I: animal and clinical studies. Neurosci. Behav. Rev. 11:341–352; 1987.
11. Cole, B. J.; Robbins, T.W. The effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: implications for theories of selective attention and arousal, Behav. Brain Res. 33, 165–179. 1989.
12. Cole, B. J.; Robbins, T. W. Forebrain norepinephrine: role in controlled information processing in the rat. Neuropsychopharmacology 7:129–142; 1992.
13. Correa-Sales, C.; Rabin, B. C.; Maze, M. A hypnotic response to dexmedetomidine, an α_2 agonist, is mediated in the locus coeruleus in rats. Anesthesiology 76:948–952; 1992.
14. Everitt, B. J.; Robbins, T. W.; Selden, N. R.Functions of the locus coeruleus noradrenergic system: a neurobiological and behav-

ioural synthesis. In: Heal, D.J.; Marsden, C. A. , eds. The pharmacology of noradrenaline in the central nervous system. Oxford: Oxford University Press; 1990:349–378.
15. Foote, S. L.; Morrison, J. H. Extrathalamic modulation of cortical function, Ann. Rev. Neurosci. 10:67–95; 1987.
16. Fritschy, J.-M.; Grzanna, R. Selective effects of DSP-4 on locus coeruleus axons: are there pharmacologically different types of noradrenergic axons in the central nervous system. In: Barnes, C.D.; Pompeiano, O. The neurobiology of locus coeruleus. Program. Brain Research, vol. 88. Amsterdam: Elsevier; 1991: 257–267.
17. Gabriel, M.; Poremba, A. L.; Ellison-Perrine, C.; Miller, J. D. Brainstem mediation of learning and memory. In: Klemm, W. R.; Vertes, R. P. Brainstem mechanisms of behavior. New York: John Wiley & Sons; 1990:269–313.
18. Hallman, H.; Jonsson, G. Pharmacological modifications of the neurotoxin action of the noradrenaline neurotoxin DSP4 on central noradrenaline neurons. Eur. J. Pharmacol. 103,:269–278; 1984.
19. Harley, C. W. A role for norepinephrine in arousal, emotion, and learning? Limbic modulation by norepinephrine and the Kety hypothesis. Prog. Neuropsychopharmacol. Biol. Psychiatry 11:419–458; 1987.
20. Heal, D. J. The effects of drugs on behavioural models of central noradrenergic function. In: Heal, D. J.; Marsden, C. A., eds. The pharmacology of noradrenaline in the central nervous system. Oxford: Oxford University Press; 1990:266–315.
21. Jonsson, G., Chemical lesioning techniques: monoamine neurotoxins. In: Björklund, A.; Hökfelt , T. ,eds. Handbook of chemical neuroanatomy, vol. I. Methods in chemical neuroanatomy. Amsterdam: Elsevier; 1983:463–527.
22. Jäkälä, P.; Sirviö, J.; Riekkinen,. Jr., P.;Haapalinna, A.; Riekkinen, P. J. The effects of atipamezole, an α_2-adrenoceptor antagonist, on the performance of rats in a 5-choice serial reaction time task. Pharmacol. Biochem. Behav. 42:903–907; 1992a.
23. Jäkälä, P.; Sirviö, J; Jolkkonen, J.; Riekkinen Jr, P.; Ascady, L. Riekkinen, P. The effects of p-chlorophenylalanine-induced serotonin synthesis inhibition on the performance of a 5-choice serial reaction time task in rats. The effects of different parametric manipulations and muscarinic blockade, Behav. Brain Res. 51:29–40; 1992b.
24. Langer, S. Z. Presynaptic regulation of monoaminergic neurons. In: Meltzer, H. Y., ed. Psychopharmacology, the third generation of progress. New York: Raven Press; 1987:151–158.
25. Lategan, A. J.; Marien, M. R.; Colpaert, F. C. Suppression of nigrostriatal and mesolimbic dopamine release in vivo following noradrenaline depletion by DSP-4: a microdialysis study, Life Sci. 50:995-999; 1992.
26. MacDonald, E.; Scheinin, H.; Scheinin, M. Behavioural and neurochemical effects of medetomidine, a novel veterinary sedative. Eur. J. Pharmacol. 158:119–127; 1988.
27. MacDonald, E.; Scheinin, M.; Scheinin, H.; Virtanen, R. Comparision of behavioral and neurochemical effects of two optical enantiomers of medetomidine, a selective alpha-2-adrenoceptor agonist, J. Pharmacol. Exp. Ther. 259:848–854; 1991.
28. McCormick, D. A. Cholinergic and noradrenergic modulation of thalamocortical processing, Trends Neurosci. 12:215–221; 1989.
29. McGaugh, J. L.Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. Annu. Rev. Neurosci. 12:255–287; 1989.
30. Mouradian, R. D.; Sessler, F. M.; Waterhouse, B. D. Noradrenergic potentiation of excitatory transmitter action in cerebrocortical slices: Evidence for mediation by an a1 receptor-linked second messenger pathway. Brain Res. 546:83–95; 1991.
31. Renouard, A.; Widdowson, S.; Millan, J. Multiple alpha2 adrenergic receptor subtypes. I. Comparison of [3H]RX821002-labelled rat Ralpha-2A adrenergic receptors in cerebral cortex to human $H_{alpha2a}$ adrenergic receptor and other populations of alpha-2 adrenergic sybtypes. J. Pharmacol. Exp. Ther. 270:946–957; 1994.
32. Riekkinen, P. J.; Sirviö, J.; Riekkinen Jr, P.; Valjakka, A.; Jäkälä, P.; Koivisto, E.; Lammintausta, R. The role of noradrenergic system in higher cerebral functions: Experimental studies about the effects of noradrenergic modulation on electrophysiology and behavior. In: Levin, E. D.; Butcher, L. L.; Decker, M. W., eds. Neurotransmitters and cognitive function. Boston: Birkhuser; 1992:91–102.
33. Riekkinen Jr., P.; Sirviö, J.; Jäkälä, P.; Lammintausta, R.; Riekkinen, P. Effect of α_2 antagonists and agonist on EEG slowing induced by scopolamine and lesion of the nucleus basalis. Neuropharmacology 29:993–999; 1990.
34. Riekkinen Jr., P.; Sirviö, J.; Lammmintausta, R.; Ekonsalo, T.; Riekkinen Sr, P. The effects of α_2-adrenergic stimulation on neocortical EEG activity in control- and 6-hydroxydopamine dorsal noradrenergic bundle-lesioned rats. Eur. J. Pharmacol. 238:263–272; 1993.
35. Robbins, T. W.; Everitt, B. J. Psychopharmacological studies of arousal and attention. In: Stahl, S. M.; Iversen, S. D.; Goodman, E. C., eds. Cognitive neurochemistry. Oxford: Oxford University Press; 1987:135–170.
36. Sara, S.; Bergis, K. O. Enhancement of excitability and inhibitory processes in hippocampal dentate gyrus by noradrenaline: A pharmacological study in awake, freely moving rats, Neurosci. Lett. 126:1–5; 1991.
37. Savola, R.; Virtanen, J.-M. Central α_2-adrenoceptors highly stereoselective for dexmedetomidine, the dextro enantiomer of medetomidine, Eur. J. Pharmacol. 195:193–199; 1991.
38. Scheinin, H.; MacDonald, E.; Scheinin, M. Behavioural and neurochemical effects of atipamezole, a novel α_2-adrenoceptor antagonist, Eur. J. Pharmacol. 151:35–42; 1988.
39. Schulteis, G.; Martinez, Jr, J. L. Peripheral modulation of learning and memory: enkephalins as a model system. Psychopharmacology 109:347–364; 1992.
40. Segal, I. S.; Vickery, R.G.; Walton, J. K.; Doze, V. A.;Maze, M. Dexmedetomidine diminishes halothane anesthetic requirements in rats through a postsynaptic alpha2 adrenergic receptor. Anesthesiology 89:818-823; 1988.
41. Segal, M.; Bloom, F. E. The action of norepinephrine in the rat hippocampus. IV. The effects of locus coeruleus on evoked hippocampal activity. Brain Res. 107:513–525; 1976.
42. Simson, P. E.; Weiss, J. M.; Alpha-2 receptor blockade increases responsiveness of locus coeruleus neurons to excitatory stimulation. J. Neurosci. 7:1732–1740; 1987.
43. Sirviö, J.; Jäkälä, P.;Mazurkiewicz, M.; Haapalinna, A.; Riekkinen, Jr., P.; Riekkinen, P. J. Dose- and parameter-dependent effects of atipamezole, an α_2-antagonist, on the performance of rats in a five-choice serial reaction time task. Pharmacol. Biochem. Behav. 45:123–129; 1993.
44. Sirviö, J.; Mazurkiewicz, M.; Haapalinna, A.; Riekkinen, Jr., P.; Lahtinen, H.; Riekkinen, P. J. The effects of selective alpha-2 adrenergic agents on the performance of rats in a 5-choice serial reaction time task. Brain Res. Bull. 35:451–455.1994.
45. Svensson, T. Peripheral, autonomic regulation of locus coeruleus noradrenergic neurons in the brain: putative implications for psychiatry and psychopharmacology. Psychopharmacology 92:1–7; 1987.
46. Uhlen, S.; Wikberg, J. E. S. Delineation of rat kidney α_2- and $\alpha_{2\beta}$-adrenoceptors with [3H]RX821002 radioligand binding: computer modelling reveals that guanfacine is a α_{2A}-selective compound. Eur. J. Pharmacol. 202:235–243; 1991.
47. Virtanen, R.; Savola, J.-M.; Saano, V.; Nyman, L. Characterization of the selectivity, specificity and potency of medetomidine as an α_2-adrenoceptor agonist. Eur. J. Pharmacol. 150:9–14; 1988.
48. Virtanen, R.; Savola, J.-M.; Saano, V. Highly selective and specific antagonism of central and peripheral α_2-adrenoceptors by atipamezole. Arch. Int. Pharmacodyn. Ther. 297:190–204; 1989.