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Effects of atomoxetine on attention in Wistar rats treated with the neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP4)

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Abstract The aim of the present study was to assess the effects of the neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP4), which allows a depletion of noradrenergic terminals in a dose-dependent manner, on attention in rats as measured using the five-choice serial-reaction time task (5CSRTT). In addition, we investigated whether the effects of DSP4 treatment can be reversed by atomoxetine. Atomoxetine is a selective noradrenaline reuptake inhibitor and has been shown to be effective in the treatment of attention deficit hyperactivity disorder. Wistar rats were trained in the 5CSRTT and treated with one of the three doses of DSP4 (10, 20 or 50 mg/kg) or saline. Following DSP4 treatment, rats were injected with three doses of atomoxetine (0.1, 0.5 or 1 mg/kg) or saline and tested in the 5CSRTT. The treatment with DSP4 caused a reduction in activity and a decline of performance in parameters related to attention in the 5CSRTT. Whether or not these impairments are due to attention deficits or changes in explorative behaviour and activity remains to be investigated. The treatment with atomoxetine had no beneficial effect on the rats' performance regardless of the DSP4 treatment. The present findings support the role of noradrenaline in modulating attentional processes and call for future studies regarding the effects of atomoxetine on attention in rats.

Keywords Atomoxetine · Attention deficit hyperactivity disorder · *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine · DSP4 · Rat

Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common psychiatric diagnoses in childhood and adolescence and can continue into adulthood (Barkley 2006; Lange et al. 2010). A multifactorial aetiology of ADHD with a complex interaction between genetic and environmental factors has been proposed (Renner et al. 2008). Many research studies have investigated neurobiological correlates of ADHD (Arnsten 2000; Faraone and Biederman 1998; Rosa Neto et al. 2002; Sontag et al. 2010; Tripp and Wickens 2009; Volkow et al. 2001), and a central dysregulation of the neurotransmitters dopamine and noradrenaline has been proposed as a pathophysiological basis of the disorder (Renner et al. 2008; Sontag et al. 2010; Thapar and Cooper 2016). This hypothesis is supported by several neurobiological, genetic and functional neuroimaging studies (Renner et al. 2008; Sontag et al. 2010; Swanson et al. 2007; Thapar and Cooper 2016; Tripp and Wickens 2009; Wankerl et al. 2014).

The prefrontal cortex and its connections with subcortical structures (Arnsten 2000, 2006; Arnsten et al. 1996; Biederman and Faraone 2005; Faraone and Biederman 1998) and the cerebellum (Castellanos et al. 1996, 2001, 2002; Durston et al. 2004) have been proposed to be involved in the pathophysiology of ADHD. Prefrontal regions regulate several aspects of behaviour including attention functions and have been shown to be influenced by noradrenaline and dopamine (Arnsten 2006; Biederman and Faraone 2005; Faraone and Biederman 1998; Robbins

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and Roberts 2007; Roberts et al. 1994; Thapar and Cooper 2016). A primarily noradrenergic dysfunction was discussed as one of the earliest models of ADHD (Mefford and Potter 1989). This was supported by the findings of animal studies, e.g. an increase in brain noradrenaline leads to more focused behaviour, while a reduction in noradrenaline increases the reaction to irrelevant stimuli (Aston-Jones et al. 1997). Pliszka et al. (1996) have postulated a dysfunction in noradrenergic and dopaminergic neurotransmission and described a posterior system, in which a noradrenergic dysfunction results in attention deficits, and an anterior system, in which a hypofunctional dopamine system results in impaired executive functions. Another hypothesis suggests a reduced noradrenaline activity inducing a changed alpha-2 receptor activity in the prefrontal cortex and resulting in a dysfunctional inhibition control in children with ADHD (Arnsten and Dudley 2005; Arnsten et al. 1996). A disturbed inhibition control induced by a decrease in noradrenaline has therefore been suggested as the main neurobiological correlate of ADHD. There are differences between the hypotheses attempting to explain the neurobiological basis of ADHD, but they have certain aspects in common, i.e. the important role of catecholamines, particularly noradrenaline, and the prefrontal cortex (Sontag et al. 2010).

Noradrenaline has been shown to be involved in the regulation of the signal-to-noise ratio (McGaugh 1989) and the facilitation of excitatory and inhibitory responses in the limbic system (Pichler and Kobinger 1985; Riekkinen et al. 1993). In addition, noradrenaline plays an important role in arousal, vigilance and responses to novel, salient stimuli (Arnsten 1997; Aston-Jones et al. 1994; Clark et al. 1987; Harley 1987; Solanto 1998). Other processes such as learning and attention are also influenced by noradrenergic activity (Cole and Robbins 1989; Coull 1994; Everitt et al. 2009; Jonge et al. 1981; Micheletti et al. 1987). Since some of these functions are known to be affected in ADHD, it is interesting to focus on noradrenaline and its possible aetiological importance in this disorder. This view is supported by the fact that compounds such as methylphenidate and atomoxetine are effective in the pharmacological treatment of ADHD and affect the noradrenergic system by reuptake inhibition of noradrenaline (Arnsten 2011; Sontag et al. 2010).

Noradrenergic functioning in the brain can be impaired by noradrenaline depletion induced by the administration of the neurotoxins 6-hydroxydopamine (6-OHDA) (Brozoski et al. 1979; Collins et al. 1998; Crofts et al. 2001; Roberts et al. 1994) or *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP4) (Cheetham et al. 1996; Fritschy and Grzanna 1991; Ross and Stenfors 2015). A depletion of noradrenaline in neonatal rats following the administration of 6-OHDA in combination with a selective dopamine

transporter inhibitor (Teicher et al. 1986) has been shown to induce motor hyperactivity (Raskin et al. 1983), learning deficits (Roberts et al. 1976) and attention deficits (Carli et al. 1983). The noradrenergic depletion induced by DSP4 has several advantages. DSP4 can be administered systemically, is effective in a dose-dependent manner and selective for the noradrenergic terminals of the locus coeruleus (Fritschy and Grzanna 1991; Ross and Stenfors 2015). Previous studies have assessed behavioural aspects of noradrenergic depletion following DSP4 administration. While some authors have reported impaired spatial working memory after DSP4 administration in rats (Compton et al. 1995; Sontag et al. 2008; Wenk et al. 1987), others were unable to reveal any deficits (Al Zahrani et al. 1997; Benloucif et al. 1995; Langlais et al. 1993; Sontag et al. 2011). These inconsistent findings and the fact that the effect of a systemic noradrenergic depletion on attention has not sufficiently been investigated emphasise the need for further research. In this context, it is also interesting to investigate different doses of DSP4 which have been shown to induce different degrees of noradrenergic depletion (Cheetham et al. 1996).

The aim of the present study was to elucidate the role of central noradrenaline regarding attentional aspects of cognitive performance in rats by using an established test paradigm for the assessment of attention in rodents, i.e. the five-choice serial-reaction time task (5CSRTT). We have assessed attentional processes following a systemic administration of the neurotoxin DSP4 and the influence of the selective noradrenaline reuptake inhibitor atomoxetine on DSP4-treated rats.

Methods

Animals and feeding procedure

All experiments were performed in accordance with the national laws (German law on Protection of Animals) and the principles of laboratory animal care (NIH publication No. 86-23, revised 1996). The experimental protocol was approved by the District Government of the Oberpfalz/Bavaria (Permit number 54-2531.1-14/07). The rats were handled according to the guidelines of the Federation for European Laboratory Animal Science Associations (FELASA).

Forty-eight male Wistar rats (Charles River Laboratories, Sulzbach, Germany) aged 7 weeks (body weight approximately 300 g) at the beginning of the experiment were used. The entire experiment (training, recovery period after DSP4 administration and the testing phase under atomoxetine conditions) lasted 14 weeks. The rats were housed in standard cages under standard animal laboratory

conditions (12:12 h light/dark cycle, room temperature 22 °C, humidity 50%) in the animal laboratories of the University of Regensburg. All treatments, trainings and tests were performed during the light phase between 9 a.m. and 4 p.m. Access to food was restricted since the behavioural paradigm used in this study (5CSRTT) is based on food reinforcement. Water was provided *ad libitum*. After the training or testing procedures, the rats had free access to food for 1 h a day. The rats' weight was carefully controlled, and a weight reduction of more than 5% was avoided in order to prevent stress (Bear 1999; Deroche et al. 1995) and subsequent changes in the dopaminergic system (Pothos et al. 1995). After the DSP4 administration, the rats were given a recovery period of 2 weeks, during which time they were monitored daily for health concerns and body weight. Body weight was assessed in order to avoid a reduction in body weight following the administration of DSP4. In case of weight loss, the rats were fed individually with food mash. No experiments or tests were conducted during this recovery period. After the experiments, rats were sacrificed using carbon dioxide.

The five-choice serial-reaction time task

The experiment was performed using four ventilated wooden chambers (Campden Instruments, Loughborough, Leicestershire, England) containing a stainless steel chamber (26 cm × 26 cm × 30 cm height). The steel chambers were lightened by 3-watt light bulbs. Each chamber was equipped with five holes, which were arranged horizontally in the curved rear wall. The holes were 2 cm above the chamber floor (stainless steel grid), each hole had a diameter of 2 cm and adjacent holes were 6 cm apart. In each hole, an infrared photocell was installed in order to detect a nose poke response of the rat to the hole. In addition, each hole was equipped with a standard light bulb (3 W). The animals were required to respond correctly to a stimulus by a nose poke into one of the five holes. A stimulus was defined as the illumination of a hole by the light bulb, and only one hole at a time could be illuminated. A correct response was rewarded with a food pellet (45 mg dustless sucrose pellets, Bio-Serv, Frenchtown, New Jersey, USA) which was dispensed into a food tray at the front wall (opposite the holes). False responses, premature responses or omissions were punished with a 5-s period of darkness.

The behavioural paradigm consisted of three phases. In the habituation phase, the ambient light was permanently turned on, 10 pellets were baited in the food tray and one pellet was placed in each illuminated hole. The rats were required to habituate to the boxes for 30 min a day. The habituation phase was finished when all pellets were found and collected, which was accomplished within two

consecutive days. In the training phase, the rats were required to learn to respond correctly to the stimulus (i.e. random illumination of a hole, once per trial) in order to obtain a food pellet. The stimulus duration was gradually reduced when a rat responded correctly—within one training session of 30 min—in at least 80% of the trials (number of correct trials/total correct and false responses expressed as percent), and the omission rate was less than 20% (number of trials missed/total trials completed expressed as percent). The stimulus duration lasted from 60 s (training level 1) to 1.5 s (final training level). All other parameters were kept constant during the training phase (inter-trial interval ITI of 5 s). In the final (testing) phase, the stimulus duration was 1.5 s and the test sessions were similar to the training sessions except that the ITIs varied randomly between 0.5, 1.5, 3 and 4.5 s. Each ITI occurred 25 times within one session. The order in which the groups were tested was randomised in all phases.

Training and DSP4 administration

The training was performed with untreated rats in order to avoid any effects of the DSP4 treatment on learning. The animals were trained on five consecutive days per week for 8 weeks (40 sessions). After the training phase, the rats were divided randomly into four groups of 12 rats each and were treated with one dose of DSP4 (10, 20 or 50 mg/kg body weight) or saline. The DSP4 doses used in the present study are based on the findings of Cheetham et al. (1996). According to these authors, the DSP4 doses used cause a noradrenergic depletion in the rat brain in a linear fashion by 51, 73 or 100%, induced by the selective destruction of the noradrenergic terminals originating in the locus coeruleus. DSP4 (Sigma-Aldrich, Schnelldorf, Germany) was dissolved in saline, the volume injected was calculated individually (1 ml/kg body weight) and both DSP4 and saline were injected intraperitoneally.

A period of 2 weeks followed the DSP4 administration in order to allow for the recovery of the peripheral noradrenaline levels. During this period, the rats were checked for body weight and general health once a day. The feeding procedure was less restrictive to prevent additional stress, and the rats had free access to food during daytime. Rats were fed with food mash when a weight reduction of more than 5% was observed.

Testing and treatment with atomoxetine

During the test phase, the rats were tested twice a week (Tuesday and Friday). On the day prior to testing, rats were trained using the stimulus duration of the final training level to guarantee optimal performance of the animals. In the test phase, the rats were treated with atomoxetine. On

testing days, the rats were injected with saline (control) or atomoxetine at a dose of 0.1, 0.5 or 1 mg/kg body weight (Sigma-Aldrich, Schnelldorf, Germany) 20 min prior to testing. Atomoxetine doses were used on the basis of previous findings (Bari et al. 2009; Jentsch et al. 2009; Koda et al. 2010; Liu et al. 2015; Navarra et al. 2008; Robinson et al. 2008). Atomoxetine was dissolved in saline, and the volume injected was determined individually (1 ml/kg body weight). Both atomoxetine and saline were administered intraperitoneally. Each rat received one dose of atomoxetine twice a week and was exposed to all doses and the control condition. The testing period lasted 4 weeks. Saline and the three atomoxetine doses were administered in a balanced manner in order to avoid systematic carry-over effects.

Statistical analysis

The following parameters were analysed: (1) number of correct responses, (2) number of false responses, (3) number of missed responses (omissions), (4) percentage of correct responses (i.e. number of correct responses/total correct and false responses expressed as percent) and (5) percentage of missed responses (i.e. total number of missed responses/total trials completed expressed as percent). In addition, the following parameters were analysed for the comparisons between the DSP4 treatment groups: (6) number of front beam breaks and (7) number of back beam breaks. To compare the differences between the DSP4 groups and between the atomoxetine conditions, a mean value of performance on the two test days per week was calculated for each animal. All findings concerning group differences and comparisons between the atomoxetine conditions within each group are expressed as means $\pm$ standard errors. The statistical analysis of differences between the DSP4 groups was performed using the Mann–Whitney *U* test (between-subject design). The atomoxetine doses within each DSP4 group were compared using the Wilcoxon test. An α level of 0.05 was applied. All statistical analyses were performed using the Statistical Package for Social Sciences 19.0 (SPSS) for Windows.

Results

Comparisons between the DSP4 groups in the atomoxetine_control condition (for details see Table 1)

All means (M) and standard errors (SE) of the percentage and number of correct responses, the percentage and number of missed responses and the number of false

responses of the DSP4 groups in the atomoxetine conditions are given in Table 1. With regard to comparisons between the DSP4 groups in the atomoxetine_control condition, statistically significant differences were found regarding both correct responses and false responses. The DSP4_50 mg/kg group made significantly fewer correct responses than the DSP4_control group ($p = 0.036$; $Z = -2.094$) and the DSP4_10 mg/kg group ($p = 0.033$; $Z = -2.136$). Furthermore, the DSP4_50 mg/kg group made significantly fewer false responses compared to the DSP4_control animals ($p = 0.03$; $Z = -2.171$). None of the remaining comparisons between the groups reached statistical significance.

The administration of the high DSP4 dose (50 mg/kg) significantly reduced the numbers of front beam breaks compared to the DSP4_control group and the DSP4_10 mg/kg group (DSP4_50 mg/kg (M $\pm$ SE): 150.41 ± 8.81 ; compared with DSP4_control (M $\pm$ SE): 229.5 ± 6.24 ; $p = 0.002$; $Z = -3.109$; compared with DSP4_10 mg/kg (M $\pm$ SE): 222.09 ± 5.57 ; $p = 0.003$; $Z = -2.922$). The comparisons between the middle DSP4 dose (DSP4_20 mg/kg (M $\pm$ SE): 221.9 ± 7.69) and the other DSP4 doses and all the remaining comparisons between the DSP4 treatment groups concerning the number of front beam breaks failed statistical significance.

The treatment with 50 mg/kg DSP4 caused a significant decrease in the numbers of back beam breaks in comparison with the DSP4_control and DSP4_10 mg/kg groups (DSP4_50 mg/kg (M $\pm$ SE): 155.36 ± 12.27 ; compared with DSP4_control (M $\pm$ SE): 248.79 ± 7.14 ; $p = 0.008$; $Z = -2.646$; compared with DSP4_10 mg/kg (M $\pm$ SE): 219.41 ± 7.99 ; $p = 0.045$; $Z = -2.003$). All other comparisons concerning the number of back beam breaks were not statistically significant [DSP4_20 mg/kg (M $\pm$ SE): 214.5 ± 9.84]. The numbers of front and back beam breaks are presented in Fig. 1.

Differences between atomoxetine doses within the DSP4 groups (for details see Table 1)

Within the DSP4_control group, the administration of 1 mg/kg atomoxetine led to a significant decrease in correct responses in comparison with the atomoxetine_control condition ($p = 0.018$; $Z = -2.356$). In addition, the animals of the DSP4_control group made statistically significant fewer false responses in the atomoxetine_0.5 mg/kg condition compared to the atomoxetine_control condition ($p = 0.008$; $Z = -2.637$).

The DSP4_10 mg/kg group showed significantly more correct responses when treated with 1 mg/kg atomoxetine compared to the dose of 0.5 mg/kg ($p = 0.05$; $Z = -1.956$). No other difference between the atomoxetine doses reached statistical significance.

Table 1 Performance in the 5CSRTT with regard to atomoxetine treatment in the DSP4 groups (M ± SE)

	Atomoxetine control	Atomoxetine 0.1 mg/kg	Atomoxetine 0.5 mg/kg	Atomoxetine 1 mg/kg
DSP4_control				
Number of correct responses	28.38 ± 1.45	23.75 ± 1.93	24.67 ± 1.59	23.63 ± 1.91 ^a
Number of false responses	3.13 ± 0.35	1.96 ± 0.19	1.75 ± 0.23 ^a	2 ± 0.3
Number of missed responses	11.42 ± 0.67	12.63 ± 0.74	14.38 ± 0.77	12.79 ± 0.65
Percentage of correct responses	87.11 ± 2.19	88.3 ± 2.1	93.22 ± 0.79	82.58 ± 2.98
Percentage of missed responses	33.95 ± 2.82	41.61 ± 3.22	39.02 ± 2.32	44.03 ± 3.51
DSP4_10 mg/kg				
Number of correct responses	27.86 ± 1.13	23.14 ± 1.71	21.55 ± 1.45	27.23 ± 1.61 ^b
Number of false responses	2.36 ± 0.27	1.55 ± 0.12	2 ± 0.23	2.27 ± 0.23
Number of missed responses	14.27 ± 0.75	13.59 ± 0.43	13.68 ± 0.75	15.68 ± 1.08
Percentage of correct responses	92.18 ± 0.87	91.41 ± 0.93	87.39 ± 2.61	92.74 ± 0.8
Percentage of missed responses	35.75 ± 1.87	43.51 ± 2.47	44.66 ± 2.57	37.61 ± 3
DSP4_20 mg/kg				
Number of correct responses	25.95 ± 1.9	24.91 ± 1.72	21.14 ± 1.16	18.91 ± 1.67
Number of false responses	2.82 ± 0.28	2.45 ± 0.21	2.18 ± 0.15	1.91 ± 0.28
Number of missed responses	14.55 ± 0.9	15.45 ± 0.94	15.36 ± 0.69	15.41 ± 0.88
Percentage of correct responses	89.83 ± 0.9	81.16 ± 2.93	89.69 ± 1.12	86.09 ± 1.39
Percentage of missed responses	39.11 ± 3.35	42.3 ± 3.08	43.71 ± 2.02	50.78 ± 3.26
DSP4_50 mg/kg				
Number of correct responses	17.05 ± 1.87 ^{1, 2}	19.85 ± 1.76	13.94 ± 1.77	18.17 ± 1.86
Number of false responses	1.45 ± 0.19 ¹	1.2 ± 0.16	1.17 ± 0.14	1.33 ± 0.1
Number of missed responses	14.18 ± 1.41	15.45 ± 1.42	11.67 ± 1.25	12.11 ± 0.82
Percentage of correct responses	78.98 ± 4.56	91.99 ± 1.41	81.72 ± 3.35	91.37 ± 0.77
Percentage of missed responses	51 ± 3.3	45.21 ± 2.62	53.42 ± 2.34	44.11 ± 2.56

¹ Compared with DSP4_control² Compared with DSP4_10 mg/kg^a Compared with atomoxetine control^b Compared with atomoxetine 0.5 mg/kg^{1, 2, a, b} $p < 0.05$

All remaining differences between the atomoxetine conditions within the DSP4_20 mg/kg group and the DSP4_50 mg/kg group failed statistical significance (means and standard errors are presented in Table 1).

Discussion

The first aim of the present study was to elucidate the role of noradrenaline in attentional processes in rats as assessed by the 5CSRTT. At first glance, the results suggest an impairment in regard to attention parameters in the 5CSRTT. The number of correct responses decreased in rats treated with DSP4. This effect tended to be dose-dependent, although only the difference between the DSP4_50 mg/kg group and the DSP4_control group was statistically significant. Moreover, DSP4-treated rats

missed more signals in comparison with the DSP4_control group. However, this comparison failed to be statistically significant. Both parameters suggest that attentional processes were negatively affected by the DSP4-induced depletion of noradrenergic terminals. However, there are some contradictory results, i.e. there were no statistically significant differences between the DSP4 groups concerning the response accuracy as operationalised by the percentage of correct responses in previous studies (e.g. Carli et al. 1983; Ruotsalainen et al. 1997). The latter results are in accordance with previous findings based on the 5CSRTT. Carli et al. (1983) found that a noradrenergic depletion caused by 6-OHDA did not affect attention, since the choice accuracy was unaltered following the treatment, at least when the inter-trial intervals (ITIs) did not vary. In this study, the authors noticed the importance of varying the ITIs because their treatment caused significant changes

Fig. 1 Number of front and back beam breaks for DSP4 groups ($M \pm SE$; $*p \leq 0.05$)

in choice accuracy when the stimulus appearance was unpredictable (variable ITIs). Another study by Ruotsalainen et al. (1997) also reported that a noradrenergic depletion by DSP4 did not affect the performance of rats in the 5CSRTT, i.e. choice accuracy was not altered after DSP4 administration (Ruotsalainen et al. 1997). Liu et al. (2015) have recently investigated the effects of noradrenergic depletion by DSP4 on attention and impulsivity as measured by the 5CSRTT in rats treated with saline or atomoxetine (Liu et al. 2015). They found a reduction in impulsive responses and perseverations in the atomoxetine-treated rats, while the pre-treatment with DSP4 had no influence on these results (Liu et al. 2015). In regard to the parameters associated with attention (i.e. percentage of correct responses, percentage of omissions), the authors found no differences between the DSP4-treated and control groups (Liu et al. 2015). The same behavioural paradigm was used both in the present experiment and in previous studies. However, there are some differences. Carli et al. (1983) used a different method of noradrenergic depletion with a lesion of the dorsal forebrain bundle being induced by 6-OHDA. Beside the fact that three different doses of DSP4 were used in the present experiment in contrast to one dose chosen by Ruotsalainen et al. (1997), there is another important difference between these studies. Ruotsalainen et al. (1997) used a fixed ITI of 5 s, while variable ITIs were used in the present experiment. In order to assess attention deficits, it is important, as was shown by Carli et al. (1983), that the stimulus presentation is unpredictable. Consequently, Liu et al. (2015) used variable ITIs, but they used only the maximal dose of DSP4 (i.e. 50 mg/kg) in comparison with sham-treated rats (Liu et al. 2015). There are some similarities between the present experiment

and the study performed by Liu et al. (2015). For example, the high dose of DSP4 and the pre-treatment with DSP4 were the same. In both studies, the rats were trained before the treatment with DSP4. Although variable ITIs were used in both studies, there is a remarkable difference. The ITIs varied from 0.5 to 4.5 s in the present study, while Liu et al. (2015) used longer ITIs (i.e. 3, 5 and 7 s) which are more suitable to measure impulsivity. Despite the experimental differences between the previous and present experiments, the findings concerning response accuracy could be replicated in the present study.

In this study, the rats of all DSP4 groups made fewer false responses compared to the DSP4_control group. Moreover, the comparison between the DSP4_control group and the DSP4_50 mg/kg group reached statistical significance. This contradicts the effects of DSP4 treatment on the number of correct responses and the number of omissions and disagrees with previous findings showing some cognitive impairment caused by noradrenergic depletion (Brozoski et al. 1979; Carli et al. 1983; Compton et al. 1995; Coull 1994; Roberts et al. 1976; Wenk et al. 1987). On closer examination, the present data show that the DSP4-treated groups in general and the high DSP4 dose in particular, by and large, made fewer responses (i.e. correct responses and false responses) than the DSP4_control rats. In addition, the DSP4-treated groups missed more signals than the DSP4_control animals (not significant). Taken together, the present data indicate a decrease in general activity rather than the presence of attention deficits as measured using the 5CSRTT following the treatment with DSP4. This is supported by another parameter measured by the 5CSRTT system. Although the 5CSRTT does not provide a specific parameter for motor

activity, the overall activity is measured by the frequency of front and back beam breaks.

Interestingly, the DSP4-treated groups showed a dose-dependent reduction in the numbers of both front beam breaks and back beam breaks, although only the differences between the DSP4_control group and the DSP4_10 mg/kg group as well as the difference between the DSP4_control group and the DSP4_50 mg/kg group reached statistical significance. This indicates a decrease in overall activity following DSP4 administration.

This explanation is supported by the findings of Jones and Hess (2003) who investigated the role of noradrenaline with regard to the hyperactivity of the coloboma mutant mouse, an animal model of ADHD, by treating the animals with DSP4. The depletion of noradrenergic terminals by DSP4 reduced the motor hyperactivity in coloboma mutant mice (Jones and Hess 2003). This is in accord with the observations of other authors who reported a reduced explorative behaviour as a consequence of DSP4 administration (Delini-Stula et al. 1984; Harro et al. 1995; Skrebuhova et al. 1999) and a reduced hyperactivity as described above (Bruno et al. 2007). Liu et al. (2015) presented no activity data as measured by the front and back beams, and it is, therefore, impossible to compare the two studies regarding activity/motivation. The question of whether or not the depletion of noradrenaline following the administration of DSP4 induces attention deficits or a decrease in activity, possibly associated with altered motivation, cannot be answered.

The second aim of the present experiment was to investigate the influence of the selective noradrenaline reuptake inhibitor atomoxetine on DSP4-treated rats. The administration of atomoxetine had no beneficial effect on the performance of the rats, regardless of the DSP4 dose used. The findings of Cheetham et al. (1996) suggest that the administration of DSP4 at a dose of 50 mg/kg destroys 100% of noradrenergic terminals. It is therefore not surprising that atomoxetine did not affect the performance of the DSP4_50 mg/kg group. However, atomoxetine had no effect on the other DSP4 groups either. This is remarkable because well-established and effective doses of atomoxetine were used (Bari et al. 2009; Jentsch et al. 2009; Koda et al. 2010; Robinson et al. 2008). The main difference between previous findings and the present results is the noradrenergic depletion. With the exception of the study of Liu et al. (2015), atomoxetine has not previously been used in DSP4-treated animals. Interestingly, Liu et al. (2015) found only significant effects in parameters associated with impulsivity (i.e. premature responses, perseverations), which was not discussed in the present study. In regard to parameters of attention, these authors found no effects on the percentage of correct responses and a remarkable but non-significant effect on the percentage of omissions. As

mentioned above, there are both similarities and differences between the present experiment and the study conducted by Liu et al. (2015). For example, different ITIs, stimulus durations and pharmacological doses were used. Nevertheless, the present data largely endorses the findings of Liu et al. (2015). In both studies, most effects failed statistical significance, although there are some indications that the treatment with atomoxetine had a detrimental effect in DSP4-pretreated rats.

Possible explanations of our data include the following. (1) On the basis of the findings of Cheetham et al. (1996), one can assume that the DSP4 doses used in the present study reduced noradrenaline in the cortex of rats in a linear fashion. One might conclude that the depletion by DSP4 was too severe to be countered by the atomoxetine doses used. (2) It is possible that the atomoxetine doses used were too small in the DSP4-treated groups. Dose-response studies will be needed to clarify this issue. (3) Finally, one might conclude that no functional relationship exists between central noradrenaline and the behaviour measured in the present study. Both the results discussed above concerning treatment with DSP4 and previous findings regarding DSP4 and atomoxetine indicate that a manipulation of noradrenergic system affects behavioural aspects as measured in this experiment (Bari et al. 2009; Carli et al. 1983; Compton et al. 1995; Coull 1994; Jentsch et al. 2009; Koda et al. 2010; Liu et al. 2015; Roberts et al. 1976; Robinson et al. 2008; Sontag et al. 2008; Wenk et al. 1987).

In the DSP4_control group, an improvement in performance following atomoxetine treatment was expected, not least because previous publications suggest a beneficial effect of atomoxetine (Bari et al. 2009; Jentsch et al. 2009; Koda et al. 2010; Liu et al. 2015; Robinson et al. 2008). Interestingly, no beneficial effect could be observed in the DSP4_control group without noradrenergic depletion. Taken together, these data suggest that the treatment with atomoxetine caused a slight deficit in attentional functions. Some authors have previously reported an increase in response accuracy or correct responses in signal-reaction paradigms (Bari et al. 2009 using a stop-signal task; Jentsch et al. 2009 using a lateralised reaction time task; Navarra et al. 2008 using the 5CSRTT; Robinson et al. 2008 using a stop-signal task), while others did not find any effect following atomoxetine administration (Koffarnus and Katz 2011; Robinson et al. 2008, all using the 5CSRTT). The present data suggest no effect (response accuracy) or a minor negative effect (absolute numbers of correct and missed responses) of atomoxetine on attention. These results agree with previous findings suggesting that atomoxetine has no effect on these parameters (Koffarnus and Katz 2011; Liu et al. 2015; Robinson et al. 2008) and disagree with studies reporting a beneficial effect of atomoxetine (Navarra et al. 2008). The ambiguity of previous

and present findings is remarkable, not least because the studies mentioned above used the same paradigm (5CSRTT) and most of them used similar doses of atomoxetine. However, the present data also suggest a beneficial effect of atomoxetine, since the number of false responses was reduced following the administration of atomoxetine. This result is in line with previous studies which used various signal-reaction paradigms including the 5CSRTT. Taken together, both previous findings and the present results are not conclusive and call for further investigation.

In conclusion, the present findings showed that the administration of DSP4 leads to marked impairments in cognitive performance as measured with the 5CSRTT. It remains to be investigated whether or not these impairments are due to attention deficits or changes in explorative activity. The administration of atomoxetine had no effect on the performance of DSP4-treated rats regardless of the DSP4 doses used. The most likely explanation for this observation is that the atomoxetine doses were too small. It would therefore be useful to investigate the effects of higher doses of atomoxetine in noradrenaline depleted animals. Finally, the administration of atomoxetine to drug-naïve rats caused beneficial, detrimental or no systematic effects on behaviour as measured by the 5CSRTT. These findings call for further investigations regarding atomoxetine and attentional processes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All experiments were performed in accordance with the national laws (German law on Protection of Animals) and the principles of laboratory animal care (NIH publication No. 86-23, revised 1996). The experimental protocol was approved by the District Government of the Oberpfalz/Bavaria (Permit number 54-2531.1-14/07). The rats were handled according to the guidelines of the Federation for European Laboratory Animal Science Associations (FELASA).

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