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Effects of clonidine injections into the bed nucleus of the stria terminalis on fear and anxiety behavior in rats

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Abstract

Emotions such as fear and anxiety are mediated by a neural network containing nuclei like the amygdala, the bed nucleus of the stria terminalis and the periaqueductal gray. Noradrenaline is a neurotransmitter closely connected with the processing of stimuli eliciting these emotions. The bed nucleus of the stria terminalis contains the highest density of noradrenaline within the brain. In the present study, we investigated effects of injections of the noradrenergic α_2-adrenoceptor agonist clonidine into the bed nucleus of the stria terminalis on learned and unlearned fear (anxiety) in rats on different animal models of fear and anxiety: acquisition and expression of fear-potentiated startle, sensitization of the acoustic startle response by foot shocks and light-enhanced startle. Clonidine injections disrupted acquisition and expression of fear-potentiated startle, as well as light-enhanced startle, whereas sensitization was not affected. These results indicate that noradrenaline within the bed nucleus of the stria terminalis mediates both fear and anxiety. We suggest that there is rather a neurochemical than a neuroanatomical dissociation between learned fear and anxiety as hypothesized by Walker and Davis (Walker, D.L. and M. Davis, 1997b, Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear, J. Neurosci. 17, 9375–9383.).

Keywords: Fear conditioning; Fear-potentiated startle; Foot shock; Light-enhanced startle; Sensitization; Noradrenaline

1. Introduction

Anxiety disorders, such as general anxiety disorder, various phobias and post-traumatic stress disorder, belong to the most common psychiatric disorders in industrial nations. The investigation of the neural basis of fear and anxiety is a prerequisite to develop new strategies for the pharmacological treatment of these disorders. There is good evidence for a general distinction between learned fear and unlearned fear (anxiety) as summarized in Davis (1997). Learned fear can be regarded as a response to an explicit threatening stimulus, e. g. escape or avoidance. Unlearned fear or anxiety is considered as a more general and long-lasting state of distress elicited by less explicit, more generalized cues. Unlearned fear involves physiological arousal but often no concrete functional behavior like escape or avoidance (Lang et al., 2000). Taken together, learned fear can be regarded as a conditioned response, whereas unlearned fear or anxiety is an innate response (Davis and Shi, 1999; Walker and Davis, 1997b; Walker et al., 2003).

Walker and Davis (1997b) suggested that there is a neuroanatomical distinction between fear and anxiety. After infusions of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide (NBQX) into the central nucleus of the amygdala, fear-potentiated startle was blocked but light-enhanced startle, an animal model for anxiety, was not affected. Infusions of NBQX into the bed nucleus of the stria terminalis did not interfere with the fear-potentiated startle, but light-enhanced

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startle was blocked. NBQX in the basolateral nucleus of the amygdala blocked fear-potentiated started as well as light-enhanced startle. Thus, learned fear seems mainly to be mediated by the central nucleus of the amygdala, whereas the bed nucleus of the stria terminalis seems to mediate unlearned fear (anxiety). This was partly supported by a study of Fendt et al. (2003) demonstrating that the bed nucleus of the stria terminalis but not the central and lateral nuclei of the amygdala is involved in unlearned fear behavior induced by the presentation of predator odor (here: trimethylthiazoline, the major component of the anal gland secretions of the red fox).

The bed nucleus of the stria terminalis has the highest concentration of noradrenaline within the brain (Brownstein and Palkovits, 1984; Fuxe, 1965; Kilts and Anderson, 1986). During stress, the level of noradrenaline in the bed nucleus of the stria terminalis is highly increased (Delfs et al., 2000; Fuentealba et al., 2000; Pacak et al., 1995; Wang et al., 2001). Noradrenaline transmission in the bed nucleus of the stria terminalis is mainly regulated by α_2-adrenoceptors (Forray et al., 1997; 1999; Palij and Stamford, 1993). Specific α_2-adrenoceptor agonists, like clonidine or 5-bromo-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (UK 14304), decrease extracellular levels of noradrenaline (Fuentealba et al., 2000; Palij and Stamford, 1993). Furthermore, it was shown that noradrenaline in the bed nucleus of the stria terminalis also inhibits glutamate release through α_2-adrenoceptors (Forray et al., 1999) indicating that noradrenaline within the bed nucleus of the stria terminalis maintains an inhibitory tone over information flow mediated by glutamate.

In the present study, we investigated the effect of clonidine injections into the bed nucleus of the stria terminalis on learned and unlearned fear. We used different animal models such as acquisition and expression of fear-potentiated startle (Fendt and Fanselow, 1999), sensitization of startle by acute foot shocks (Davis, 1989), a fast context conditioning (Kiernan et al., 1995; Pilz, 1996; Richardson and Elsayed, 1998) for learned fear, and light-enhanced startle (Walker and Davis, 1997a) for unlearned fear (anxiety).

2. Materials and methods

2.1. Animals

Eighty-nine male Sprague–Dawley rats (Charles River, Sulzfeld, Germany) were used, weighing 175–300 g at the time of surgery. The rats were housed in groups of 4–6 animals and maintained on a 12 h light/dark cycle (light on at 07:30 AM) with 15 g/rat/day rat chow and water ad libitum.

The present experiments were performed in accordance with ethical guidelines for the use of animals in experiments and were approved by the local animal care committee (Regierungspräsidium Tübingen, ZP 5/99).

2.2. Surgery

For stereotaxic surgery, animals were anaesthetized with ketamine/xylazine (9:1; 100 mg/kg, i.p.) and placed into a stereotaxic frame with blunt ear bars. Bilateral stainless steel cannulae (diameter 0.7 mm) aiming at the bed nucleus of the stria terminalis (0.3 mm caudal, $\pm$2.9 mm lateral in a 10° angle from vertical and 7.0 mm ventral to Bregma, Paxinos and Watson, 1997) were implanted using standard stereotaxic procedures. After surgery and between the experiments, the cannulae were fitted with stylets (diameter 0.4 mm) to maintain patency. Rats were tested after full recovery.

2.3. Intracerebral injections

Immediately before fear conditioning or immediately before testing for fear-potentiated startle, sensitization of startle by foot shocks or light-enhanced startle, rats received microinjections of either the α_2-adrenoceptor-agonist clonidine (10 nmol dissolved in 0.5 µl isotonic saline) or saline alone. Infusions were made bilaterally through stainless steel injection cannulae (diameter 0.4 mm) at a rate of 0.1 µl/10s. After the infusion was completed, the injection cannulae were left in place for an additional 2 min to allow diffusion of the solution.

2.4. Apparatus and behavioral tests

2.4.1. Fear conditioning

Animals were trained in two identical dark boxes ($40\times60\times28$ cm^3). The floor consisted of a steel grid (bars 18 mm apart) through which foot shocks (0.6 mA, 0.5 s), serving as the unconditioned stimulus, could be delivered. The conditioned stimulus was a light that was produced by a 15 W white light bulb positioned at the top of each box. A training session consisted of a 5-min acclimation period and 10 light-shock pairings, with a mean interstimulus interval of 2 min (range 1.5 to 2.5 min). The foot shocks were presented during the last 0.5 s of the 3.7 s light stimulus.

2.4.2. Fear-potentiated startle test

Acoustic startle response was measured in wire mesh cages with a steel floor resting on a piezoelectric accelerometer (custom-made at the University of Tübingen). Movements of the rats resulted in voltage output changes of the accelerometer. These changes were amplified, digitized and analyzed on a computer. Test cages were located within sound-attenuated test chambers. Acoustic startle stimuli (depending on test between 90 and 105 dB sound pressure level) and a continuous white background noise (53 dB sound pressure level) were delivered by a loudspeaker located 40 cm from wire mesh cage. The presentation of the acoustic stimuli, illumination and foot shocks were controlled by a computer and an interface (Hortmann universal function synthesiser, Hortmann, Neck-

artenzlingen, Germany). Startle amplitude was defined as the largest difference in the activity measured by the accelerometer in a period of 80 ms before (spontaneous motor activity) and after the acoustic startle stimulus. Furthermore, spontaneous motor activity was measured during a 28-s time window before presentation of each startle trial.

For testing, the animals were put into little test cages ($20\times10\times12$ cm^3) which were fixed on the piezoelectric accelerometers. After a 5 min acclimation period, 10 initial startle stimuli (100 dB sound pressure level, 10 kHz, 20 ms duration, interstimulus interval: 30 s) were presented to induce a stable baseline of acoustic startle response magnitude. After that, 10 further startle stimuli alone and 10 startle stimuli presented 3.2 s after the onset of the conditioned light stimulus were given in a pseudorandomized order. The difference between the mean amplitudes on startle stimulus alone trials and conditioned startle stimulus trials is an operational measure of fear (Davis et al., 1993; Fendt and Fanselow, 1999).

The animals tested for the effects of clonidine injections on acquisition of conditioned fear received clonidine (n=14) or vehicle (n=12) infusion immediately before the training. Two days later, fear-potentiated startle was tested without any further treatment. For the animals tested for the effects of clonidine injections on expression of conditioned fear, fear conditioning training was carried out without any treatment. On the two following days, rats (n=12) received infusions of clonidine or vehicle counterbalanced across immediately before being tested for fear-potentiated startle.

2.4.3. Sensitization by foot shocks

For this procedure, a steel grid was put in the startle test cages (described above) through which foot shocks could be delivered. The test consisted of 40 acoustic startle stimuli (100 dB sound pressure level, interstimulus interval: 15 s), followed by 10 foot shocks (0.6 mA, 0.5 s, 1 Hz), concluded by another 40 acoustic stimuli to allow immediate response to foot shocks.

The rats (n=15) were tested on two consecutive days in a balanced design. That is, each rat was tested twice receiving each treatment (clonidine or saline) once. The amount of sensitization was calculated from the difference between the mean acoustic startle response magnitude on the 30 startle stimuli before and after the foot shock administration.

2.4.4. Light-enhanced startle

Illumination was provided by a 23-W white energy-saving fluorescent bulb, located 20 cm above test cage. Startle stimuli were presented with three different intensities (90, 95, and 105 dB sound pressure level) with an interstimulus interval of 30 s in a balanced irregular order with the restriction that each intensity occurred once in each block of three stimuli (see also experimental design of Walker and Davis, 1997a).

Rats received infusions immediately before placed into the dark test chamber. After 5 min acclimation time, they received 30 startle-eliciting stimuli in the dark (phase I). Thereafter, 30 further startle stimuli were presented either in dark or bright continuous illumination (phase II).

Each rat (n=21) was tested on four consecutive days under all four conditions (saline: phase II dark, saline: phase II light, clonidine: phase II dark, saline: phase II light). Phase I is tested in the dark in all four conditions. The order of treatment and session type was counterbalanced across animals. For each animal, the difference between mean startle amplitude of phase I and phase II was calculated.

2.5. Histology

After completion of tests, animals were killed by an overdose of pentobarbital (Narcoren, Rhone-Merieux, Laupheim, Germany). The brains were removed and immersed in 8%-paraformaldehyde–20%-sucrose. Coronal sections of 60 μm were cut, mounted and stained with thionine.

2.6. Statistical analysis

Statistical analyses of the data were accomplished either by t-test for dependent samples (motor activity) or by different designs of analysis of variances (ANOVA). The criterion of statistical significance was $P<0.05$. Statistical analyses were executed with STATISTICA (1999, StatSoft, Tulsa OK, USA). All graphs show group means, and error bars are ±S.E.M.

3. Results

3.1. Histology

Only animals with placement of cannulae in the ventral part of the bed nucleus of the stria terminalis close to the anterior commissure were included in data analysis (n=74). Animals with misplaced injection sites were excluded from analysis (n=15). Fig. 1 shows a typical bilateral injection site in the ventral bed nucleus of the stria terminalis.

3.2. Acquisition of fear-potentiated startle

The effects of clonidine injections into the bed nucleus of the stria terminalis on the acquisition of fear-potentiated startle are illustrated in Fig. 2. Data was analyzed by an ANOVA with treatment as between-groups factor and trial type (tone alone vs. light-tone) as within factor (repeated measure). Comparisons of the startle amplitudes after tone alone trials versus the startle amplitudes after light-tone trials were made using simple contrasts. ANOVA indicated neither a significant effect for treatment nor the startle amplitude [F's<1] but there was a significant treatment x trial type interaction [$F(1,24)=8.26$, $P<0.01$]. Further analysis

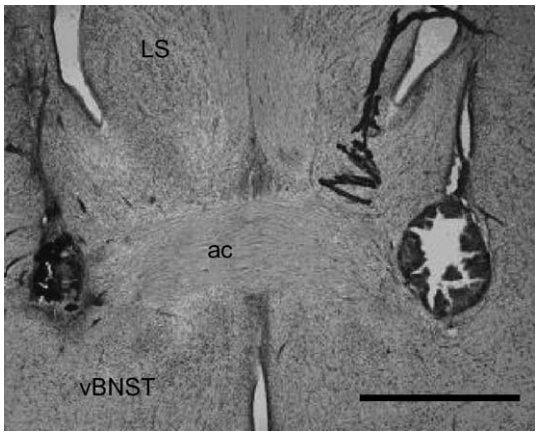

Fig. 1. Photomicrograph of a Nissl-stained coronal section showing typical bilateral injection sites into the bed nucleus of the stria terminalis. Abbreviations: ac, anterior commissure; LS, lateral septum; vBNST, ventral part of the bed nucleus of the stria terminalis; Scale bar, 1 mm.

showed, that the control group had a significant increase of the startle amplitude when startle stimulus was combined with light conditioned stimulus [$F(1,24)=9.96$, $P<0.01$], whereas in the clonidine group, this fear-potentiation was completely blocked [$F(1,24)=0.98$, $P=0.33$]. Both, baseline startle amplitude and spontaneous motor activity were not affected by clonidine injections into the bed nucleus of the stria terminalis [F's<1].

3.3. Expression of fear-potentiated startle

The effects of clonidine injections into the bed nucleus of the stria terminalis on the expression of fear-potentiated startle are illustrated in Fig. 3A. For the expression of fear-potentiated startle, we used a two-way within-subjects repeated-measure ANOVA with treatment and trial type as

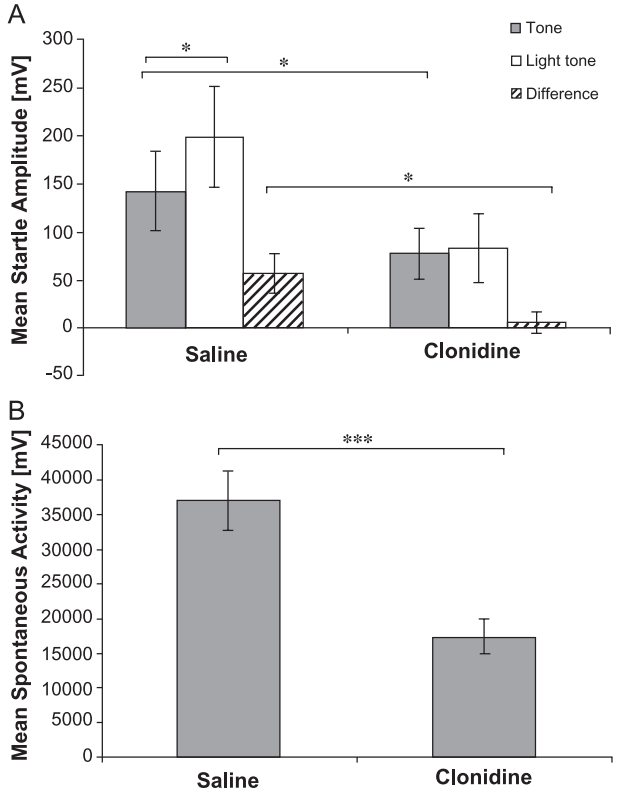

Fig. 3. (A) Bar diagram depicting the effects of clonidine injections into the bed nucleus of the stria terminalis on the expression of fear-potentiated startle. The bars represent the mean startle magnitudes (±S.E.M.) after tone alone (gray bars) and light-tone trials (white bars), as well as the difference scores (hatched bars). Clonidine injections blocked the expression of fear-potentiated startle but also decreased the baseline of startle. *$P<0.05$ (two-way ANOVA). (B) Bar diagram showing the effects of clonidine injections into the bed nucleus of the stria terminalis on spontaneous motor activity during the startle test session. Spontaneous motor activity was decreased after clonidine injections. ***$P<0.01$ (t-test).

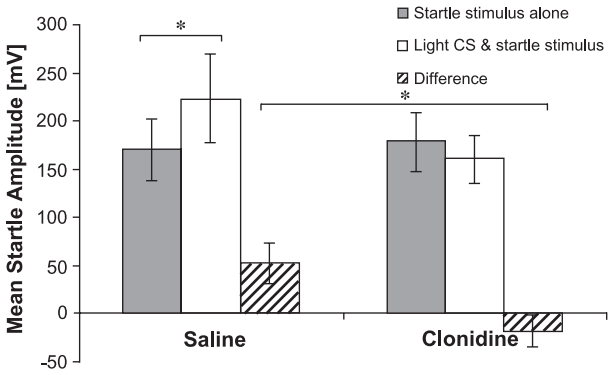

Fig. 2. Bar diagram showing the effects of clonidine injections into the bed nucleus of the stria terminalis on the acquisition of fear-potentiated startle. The bars represent the mean startle magnitudes (±S.E.M.) after tone alone (gray bars) and light-tone trials (white bars), as well as the difference scores (hatched bars). The control group had a significant increase of the startle magnitude when tone is combined with light conditioned stimulus indicating acquisition of conditioned fear after saline injections. Importantly, clonidine injections totally blocked the acquisition of conditioned fear. *$P<0.05$ (two-way ANOVA).

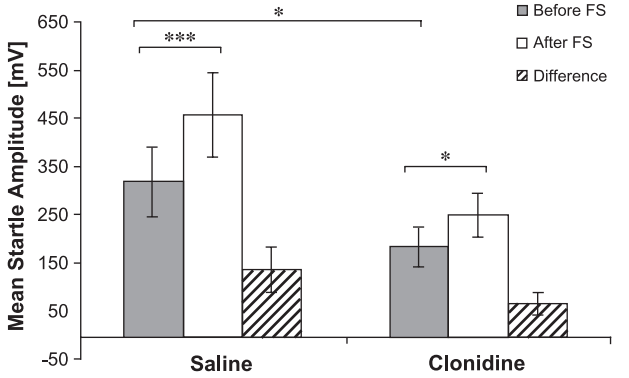

Fig. 4. Bar diagram depicting the effects of clonidine injections into the bed nucleus of the stria terminalis on foot shock sensitization of the startle response. The bars represent the mean startle magnitudes (±S.E.M.) before (gray bars) and after (white bars) foot shock administration, as well as the difference scores (hatched bars). Clonidine did not affect foot shock sensitization of the startle response indicated by a significant increase of the startle amplitude after foot shock in both groups, animals receiving saline and animals receiving clonidine injections. *$P<0.05$, ***$P<0.01$ (two-way ANOVA).

within-subjects factors. Pairwise comparisons were again done using simple contrasts. ANOVA indicated a significant effect for treatment [$F(1,11)=9.30$, $P<0.05$], as well as for the trail type [$F(1,11)=8.58$, $P<0.05$]. There was a trend for a significant interaction of treatment x trial type [$F(1,11)=4.66$, $P=0.054$]. The subsequent contrast analysis revealed a significant increase of the startle amplitude in the control group by the light conditioned stimulus [$F(1,11)=8.15$, $P<0.05$], this increase in the startle amplitude was not observed after clonidine injections into the bed nucleus of the stria terminalis [$F(1,11)=0.32$, $P=0.58$]. Admittedly, the baseline startle amplitude was decreased when clonidine injections were administered prior to testing [$F(1,11)=8.31$, $P<0.05$]. During the startle test sessions, there was also a decrease of the general motor activity confirmed by a t-test for dependent samples [$t_{(11)}=4.40$; $P<0.01$] (Fig. 3B).

3.4. Sensitization by foot shocks

The effect of clonidine injections into the bed nucleus of the stria terminalis on the sensitization of the startle response by foot shocks is shown in Fig. 4. Data for the sensitization was analyzed in the same manner as the expression of fear-potentiated startle with treatment and blocks (startle amplitude before versus startle amplitude after foot shocks) as within-subjects factors (repeated measures). ANOVA indicated a significant effect of treatment [$F(1,14)=7.56$, $P<0.05$], and a significant increase of startle amplitude after receiving foot shocks [$F(1,14)=14,24$, $P<0.01$]. Interestingly, there was no significant treatment x block interaction [$F(1,14)=2.03$, $P=0.18$]. The contrast analysis shows a significant increase of startle amplitude after receiving foot shocks with vehicle [$F(1,14)=9.02$, $P<0.01$], as well as with clonidine injections [$F(1,14)=7.28$, $P<0.05$]. Again, baseline startle amplitude was decreased after clonidine injections [$F(1,14)=5.91$, $P<0.05$].

3.5. Light-enhanced startle

The effect of clonidine injections on the light-enhanced startle is shown in Fig. 5. Data was analyzed by a three-way repeated-measure ANOVA with treatment, phase (two levels: phase I and phase II) and session type (i.e., dark→dark vs. dark→light) as within-subject factors. A post hoc comparison was made using Tukey (Honest Significant Difference) test. ANOVA revealed a significant effect of treatment [$F(1,20)=25.41$, $P<0.001$], of the phases [$F(1,20)=10.00$, $P<0.01$] and session type, i.e., the amplitude of startle was increased significantly when elicited in the presence of bright light compared to the startle amplitude elicited in the dark reflected by a significant effect of [$F(1,20)=13.50$, $P<0.01$]. Light-enhanced startle was significantly disrupted by clonidine injections into the bed nucleus of the stria terminalis indicated by significant treatment×phase×session type interaction [$F(1,20)=8.40$, $P<0.01$]. Post-hoc comparison showed that after vehicle injections, there was a significant increase of startle amplitude during the dark versus light sessions in phase II when light is present ($P<0.01$), this effect is disrupted after clonidine administrations into the bed nucleus of the stria terminalis ($P>0.9$). No change in startle amplitude is observed between phase I and phase II during the dark versus dark sessions after either treatment ($P>0.9$), but the baseline startle amplitude is again diminished after clonidine injections ($P<0.05$).

4. Discussion

In the present study, the effects of clonidine injections into the bed nucleus of the stria terminalis on four different animal models for learned and unlearned fear were investigated. Clonidine injections into the bed nucleus of the stria terminalis in the used concentrations decrease noradrenaline

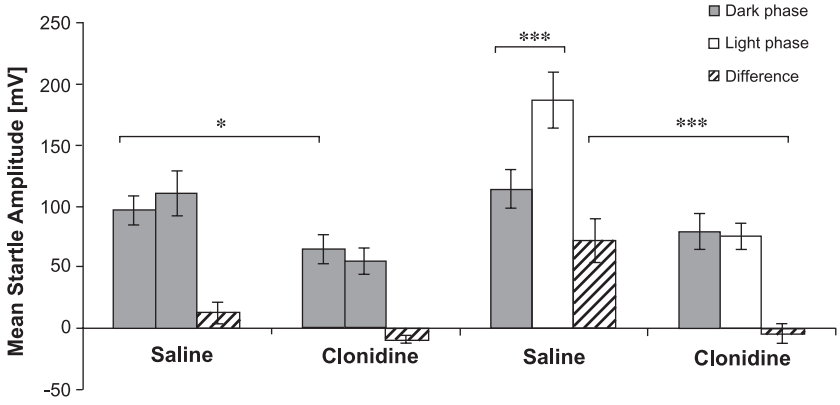

Fig. 5. Bar diagram depicting the effects of clonidine injections into the bed nucleus of the stria terminalis on light-enhanced startle. The bars represent the mean startle magnitudes (±S.E.M.) in the dark phases (gray bars) and light phases (white phases) of the light-enhanced startle test as well as the difference scores (hatched bars). Again, baseline startle magnitude was decreased after clonidine injections. In the control group, the startle magnitude is increased in the light phase indicating light-enhanced startle. This increase could not be observed after clonidine injections indicating a total blockade of light-enhanced startle. *$P<0.05$, ***$P<0.01$ (two-way ANOVA).

release within the bed nucleus of the stria terminalis (Delfs et al., 2000; Steiniger and Fendt, in preparation; Wang et al., 2001). Infusions of clonidine into the bed nucleus of the stria terminalis impaired the acquisition as well as the expression of fear-potentiated startle. Sensitization of startle response by electric foot shocks was not affected demonstrating that the ability to perceive the foot shocks was not affected by clonidine. Light-enhanced startle was completely blocked by clonidine.

Noradrenaline is an important transmitter in the processing of aversive stimuli (e.g., foot shocks or bright light for nocturnal animals) and the emotions elicited by this kind of stimuli (de Jongh et al., 2002; Walker and Davis, 1997a,b, 2002). An increased release of noradrenaline within the bed nucleus of the stria terminalis can be measured by microdialysis after aversive and stressful stimuli (Cecchi et al., 2002; Pacak et al., 1995). As a matter of fact, the bed nucleus of the stria terminalis has the highest concentration of noradrenaline in the brain (Brownstein and Palkovits, 1984; Fuxe, 1965; Kilts and Anderson, 1986). This noradrenaline is released by the terminals of fibers mainly descending from the A1 and A2 regions in the medulla oblongata (Delfs et al., 2000; Forray et al., 2000; Onaka et al., 2001; Phelix et al., 1994; Ungerstedt, 1971; Wang et al., 2001). Furthermore, a very high concentration of α_2-adrenoceptors was described (Wamsley et al., 1992). These presynaptically located receptors play an important role in the regulation of noradrenaline release (Starke, 2001). Clonidine mainly acts via the α_2-adrenoceptors and several studies showed a distinct decrease of noradrenaline release after clonidine infusions into the bed nucleus of the stria terminalis (Delfs et al., 2000; Forray et al., 1997; 1999; Fuentealba et al., 2000; Palij and Stamford, 1993; Steiniger and Fendt, in preparation; Wang et al., 2001). So, we are confident that the effects observed in the present study are due to the decreasing effects of clonidine on noradrenaline release.

One of the main effects of clonidine in the present study was a decrease in the baseline startle amplitude (experiments 2, 3, and 4). There are two possible reasons for this effect: (1) clonidine infusions into the bed nucleus of the stria terminalis have an anxiolytic effect or (2) there are some sedative side-effects. Both effects lead to a decrease in baseline startle and were previously observed after systemic or intra-amygdaloid injections of clonidine (Davis et al., 1977; Schulz et al., 2002; Spyraki and Fibiger, 1982). The question is now whether this decrease in baseline startle is based on the anxiolytic or on the sedative effect of clonidine. The fact that in the present study the amplitude of the spontaneous motor activity is decreased after clonidine injections could help to answer this question because spontaneous motor activity is also decreased by sedative drugs but usually enhanced by anxiolytic drugs (e.g., Bourin and Hascoet, 2003). So, we suggest that the observed clonidine effect on baseline amplitude is mainly due to the sedative effects of clonidine within the bed nucleus of the stria terminalis. This is supported by the observation that the clonidine effect on baseline startle amplitude and motor activity is not conditioned (experiment 1), whereas one would expect that an anxiolytic effect on baseline startle would be learned and also be observed in a following test.

In the present study, there was a clear blockade of acquisition and expression of fear-potentiated startle after clonidine infusions into the bed nucleus of the stria terminalis. It should be noted that the sensitization of the startle response by foot shocks was not affected by clonidine infusions into the bed nucleus of the stria terminalis indicating that the perception and processing of foot shocks are not disturbed by clonidine. The fact that the bed nucleus of the stria terminalis is involved in the acquisition and expression of conditioned fear is to some extent contradictory to the results of Walker and Davis (1997b) demonstrating no effects on acquisition and expression of fear-potentiated startle after a blockade of AMPA receptors within the bed nucleus of the stria terminalis. Therefore, Walker and Davis suggested that the bed nucleus of the stria terminalis is not involved in the mediation of conditioned fear. This general idea is not supported by the present data. Nevertheless, it is conceivable that noradrenaline receptors within the bed nucleus of the stria terminalis are involved in conditioned fear, whereas AMPA receptors are not. This would indicate that there is rather a neurochemical than a neuroanatomical dissociation between learned and unlearned fear (as suggested by Walker and Davis, 1997b). Against this hypothesis stands the fact that chronic lesions of the bed nucleus of the stria terminalis did not affect acquisition and expression of fear-potentiated startle (Gewirtz et al., 1998). It should be noted that in this study, a very long training phase (20 sessions) was carried out. Furthermore, a mixed training and testing procedure was used so that a comparison of the data of this study with our data is very difficult. However, further works should investigate the effects of lesions or blockade of other transmitters within the bed nucleus of the stria terminalis on conditioned fear.

In contrast to fear-potentiated startle, foot shock sensitization of the startle response is a test to investigate the *short time effects* of foot shocks. It is shown that foot shock sensitization is based on a fast association of the testing context with the foot shocks (Richardson and Elsayed, 1998). Our result that clonidine injections into the bed nucleus of the stria terminalis did not affect foot shock sensitization of the startle response confirms a previous study by Gewirtz et al. (1998). In this study, chronic lesions of the bed nucleus of the stria terminalis did not block rapid foot shock sensitization of the startle response. In contrast, the long-term sensitization of the startle response by repeated foot shock administration over many days was blocked by these lesions.

As expected, light-enhanced startle is blocked completely after clonidine injections into the bed nucleus of the stria terminalis. This supports the hypothesis that the bed nucleus

of the stria terminalis plays an important role in anxiety-like behaviors. For example, the startle amplitude is increased after injections of the anxiogenic corticotropin releasing hormone into the bed nucleus of the stria terminalis (Lee and Davis, 1997). Furthermore, injections of NBQX, a glutamate AMPA receptor antagonist, into the bed nucleus of the stria terminalis inhibit light-enhanced startle (Walker and Davis, 1997b). According to these studies, fear-like behavior is not mediated by the bed nucleus of the stria terminalis.

Forray et al. (1999) assumed that noradrenaline, which displays a tonic activity within the bed nucleus of the stria terminalis, has an inhibitory effect on glutamate transmission. The selective α_2-adrenoceptor-agonist, UK 14304, decreased the extracellular level of noradrenaline and glutamate clearly. Glutamate within the bed nucleus of the stria terminalis originates from the subicular neurons (Walaas and Fonnum, 1980), and efferents from the bed nucleus of the stria terminalis releasing ã-aminobutyric acid (GABA) project to the paraventricular nucleus of the hypothalamus (Cullinan et al., 1993). This has given rise to the hypothesis that the bed nucleus of the stria terminalis is a relay station between limbic system and the hypothalamus, which means that the bed nucleus of the stria terminalis is possibly coordinating between emotional and autonomic function in the brain (Cullinan et al., 1993).

Taken together, our results show a decrease in learned as well as in unlearned fear after clonidine injections into the bed nucleus of the stria terminalis, we assume that fear and anxiety are separated rather neurochemically than neuroanatomically as Walker and Davis (1997b) postulated.

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