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Effects of chlordiazepoxide on single-unit activity in the septal region of the freely moving rat: aversive vs. non-aversive contexts

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Abstract

Evidence suggests that stimuli that have the property of inhibiting fear in a Pavlovian fear conditioning paradigm increase cellular activity in the lateral septum, a result consistent with the idea that the lateral septum is actively involved in the inhibition of fear. The experiments reported here were designed to determine if an anxiolytic drug with fear-inhibiting properties would also increase neuronal activity in the lateral septum in a manner that might relate to its mechanism of action as an anxiolytic. An experiment was performed to compare the effects of the benzodiazepine anxiolytic chlordiazepoxide (CDP) upon single-unit activity in the septal region of the rat brain during Pavlovian aversive conditioning with the effects of CDP in a non-aversive context. During Pavlovian conditioning there was a decrease in unit activity in the more lateral regions of the septum, the dorsolateral and ventrolateral nuclei, when a stimulus signaling footshock (CS+) was presented. This conditioned suppression of unit activity was blocked by an intraperitoneal injection of CDP. Additionally, CDP increased baseline unit activity in these regions in the absence of conditioned stimuli. In the more medial regions of the septum, the intermediate lateral septum, we observed few consistent changes either to the conditioned stimuli or to the drug. In a non-aversive context CDP had either no effect at low to moderate doses, or a suppressant effect at a higher dose. The results support a fear-relief hypothesis of lateral septal functioning and suggest the lateral septum as a possible site for the anxiolytic action of benzodiazepines.

Keywords: Single unit; Benzodiazepine; Chlordiazepoxide; Pavlovian conditioning; Fear; Septum

1. Introduction

There is accumulating evidence that the lateral septum plays an important role in the inhibition of fear and anxiety. For example, in common laboratory tests of anxiety such as the Vogel (Vogel et al., 1971) water conflict test, electrical stimulation of the lateral septum has the same anti-conflict action as the anxiolytic benzodiazepines (BZDs) (Yadin et al., 1993), suggesting an anxiolytic action of septal stimulation. Electrical stimulation of the lateral septum also has a suppressing effect on species-specific defense responses, indicative of fear, induced in rats by lesions of the ventromedial nucleus (Brayley and Albert, 1977). Lesions of the lateral septum, on the other hand, appear to be pro-conflict in the conflict test, suggesting an anxiogenic effect of the lesion (Yadin et al., 1993). Consistent with an anxiogenic effect of lateral septal lesions is the finding that such lesions facilitate contextual fear conditioning in a Pavlovian model of fear conditioning (Sparks and LeDoux, 1995).

Single- and multiple-unit recording experiments in the septum also support a role for the septum in the modulation of fear conditioning (Thomas and Yadin, 1980; Thomas et al., 1991; Yadin and Thomas, 1981). The recording data are consistent with a fear-relief role for the lateral septum and a possible fear excitatory function for the medial septal regions. When animals were tested in a Pavlovian differential conditioning paradigm with an aversive US, cells in the lateral septum increased their rates of firing in the presence of stimuli that signaled relief or safety and inhibited their rates of firing in the presence of stimuli that signaled fear. Most medial cells in this study were not sensitive to the conditioning contingencies. This accords

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with data from other experiments which found virtually no associative conditioning in medial septal cells in appetitive conditioning in rats reinforced with a food US (Segal, 1973) or conditioning of the nictitating membrane response in the rabbit with an air-puff US (Berger and Thompson, 1978).

However, in contrast to the data from other laboratories we did find that there are at least a proportion of medial septal cells that do show evidence of associative conditioning. In such cells, the preponderant response appeared to be the reverse of what we observed in the lateral septum. These medial septal cells were activated by a conditioned exciter of fear. It is possible therefore that there is some contribution by the medial septum to fear conditioning and the expression of fear.

Since cells in the septum respond in a consistent manner to stimuli associated with fear and fear-relief, it would be of interest to determine if the same cells respond appropriately to pharmacological agents which affect fear. Specifically, since lateral septal cells are activated by stimuli which have fear- relief properties, it seems reasonable that anxiolytic agents such as the BZDs might have a similar effect on cells in the lateral septum. The lateral septum contains moderately high densities of GABA-linked benzodiazepine receptors (Speth et al., 1980). These receptors may be of importance in mediating the behavioral effects of BZDs since both the GABA agonist muscimol and the BZD anxiolytics midazolam and chlordiazepoxide have anxiolytic effects when administered directly into the septum (Drugan et al., 1986; Grishkat, 1991).

The behavioral effects of BZDs are substantially different in the aversive context compared to the non-aversive context. So, for instance, moderate doses of BZDs increase behavior suppressed by conditioned fear but tend not to affect behavior that hasn't been suppressed (Cook and Davidson, 1978). Accordingly, one of the key goals of the present study was determine if the effects of BZDs on septal unit activity in the two contexts parallel their effects on behavior in a manner that might account for the behavioral effects of the drug. For the aversive context we chose a Pavlovian fear conditioning paradigm where it is possible to assess the effect of BZDs both upon baseline spontaneous unit activity as well as the effect on conditioned changes in unit activity. For the non-aversive context animals were placed in the conditioning chamber but no conditioned or unconditioned stimuli were presented.

2. Methods

2.1. Subjects

Subjects were male Sprague–Dawley rats. Animals were between 90 and 110 days old and weighed approximately 350–400 g at the time of surgery. Animals were individually housed in a light and temperature controlled animal colony. The animals were maintained on a 12-h dark and 12-h light cycle and were provided with ad lib food and water throughout the experiment. Care and use of animals were approved by the Institutional Animal Care and Use Committee and experiments were carried out in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

2.2. Apparatus

The recording chamber was constructed of clear Plexiglas with a metal grid floor. The chamber measured 25×22×34 cm and was located inside an electrically shielded, wooden, sound-attenuating cubicle. The cubicle contained a 7.5-W houselight, a 15-W light used as a visual conditioned stimulus (CS), and a loudspeaker, all of which were located on the cubicle wall opposite the uncovered Plexiglas wall. The house light remained on at all times. White noise (70 db) was presented continuously except during the auditory CS which was an 800 Hz, 70 db tone. The unconditioned stimulus (US) was a 1-s footshock delivered through the grid floor. It consisted of a 100 pulse/s square wave of 1 ms duration. The US was delivered by Grass stimulator (model S44) connected in series with a constant current unit (Grass model CCU1).

Recording electrodes consisted of a bundle of eight nichrome wires each 50 microns in diameter and Teflon insulated to the tip. These wires were soldered to female Amphenol pins which were inserted into a plug on the animal's head. The wires were twisted together and cut on a 45° angle before being implanted. This configuration yields a distance of approximately 50 μm between the shortest and longest electrode tips and simulates a microdrive allowing units to be sampled at slightly different dorso-ventral coordinates.

2.3. Surgery

The animals were anesthetized with sodium pentobarbital (42 mg/kg, i.p.). All electrodes were chronically implanted using standard stereotaxic procedures. Coordinates for the lateral septum were taken from Paxinos and Watson (1998) and were as follows: 0.6 mm anterior to bregma, 0.4 mm lateral to the midline, and 6.0 mm ventral to the skull surface. The plug included a ground wire which was wrapped around one of four stainless steel screws inserted in the skull. The screws and the plug were secured to the skull surface using dental cement.

2.4. Recording procedure

Single-unit activity was recorded using a high input impedance amplification system. Field-effect transistors were cemented to the plug on the animal's head. The headstage amplifier was connected in a voltage follower configuration and recording was done differentially through electrode pairs in the site. This configuration minimizes

undifferentiated multiple unit activity virtually eliminates movement artifact as well as EMG artifact. The signal was passed through a bandpass filter (500–3000 Hz) and a high gain amplifier (A–M instruments model 1800). The final gain on the amplifiers was 10,000. The signal was monitored on a digital oscilloscope and computer screen. Recording and control of the conditioning experiment was accomplished by two computers in communication with each other. One computer served to discriminate and isolate units according to criteria described below. The signal from the amplifier was digitized and analyzed by a commercial software package which isolated the units being studied (Datawave Technologies). When a unit which satisfied the criteria was detected by the first computer a TTL pulse was delivered to a second computer which stored the data, controlled all the environmental events, and displayed an on-line, real-time histogram of unit activity during each conditioning trial. The TTL pulse also triggered the oscilloscope so that the unit waveform could be monitored, thus allowing for further monitoring of unit specificity.

2.5. Data analysis

Unit isolation was performed using cluster analysis provided by the software mentioned above. Single units were determined by applying a number of acceptance criteria which correspond to various parameters of the waveform. These criteria include spike width and height, peak magnitude, peak and valley time. In addition the unit with the identified rate and form had to be observable in a single electrode in the bundle. All acceptable units had to have a signal to noise ratio of at least 3:1. The application of these criteria as well as constant on line visual monitoring of the waveform gave reasonable assurance that single units were indeed isolated. Each of the eight electrodes in the bundle was sampled to determine if an acceptable unit could be obtained from it. Since conditioning was conducted over several sessions the acceptance parameters for unit identification were saved from session to session and applied on successive sessions. Waveforms on each day were compared in order to determine with reasonable certainty that the same unit was being sampled. If the unit was lost then the electrodes were sampled again, a search was made for new units and new acceptance parameters were defined. Units were often kept over several sessions.

2.6. Procedure

Separate groups of animal were tested in a Pavlovian aversive conditioning paradigm and in a non-aversive context.

2.6.1. Pavlovian conditioning

Twenty animals were run in this condition. Those animals with acceptable unit activity were given the following conditioning sequence. On the first day they were given a habituation session. In this session animals were presented with 40 trials in which the 10-s CSs were presented without US presentations. CS+ and CS− trials were randomly ordered according to a modified Gellermann (Gellermann, 1933) sequence in which no CS of a given type was presented on more than three consecutive trials. Intertrial intervals were randomly determined with a mean of 70 s. Subsequently, the animals received 12 sessions of Pavlovian differential conditioning. On these sessions one CS (CS+) was paired with US. For these trials the CS duration was 11 s with a 10-s CS–US interval. The CS and US overlapped for 1 s and coterminated. The other CS (CS−) was presented in the absence of the US. Animals received one session per day and each session consisted of 40 trials, 20 CS+ and 20 CS− trials. Prior to the first conditioning session the shock intensity was adjusted for each animal so that it produced a flinch with minimal vocalization. This shock intensity was used throughout the remainder of the conditioning sessions. The magnitude of the footshock for all animals ranged from 0.6 to 1.0 ma. For half of the animals the tone served as CS+ and the light as CS−. For the other half the light served as CS+ and the tone as CS−. On each trial unit activity was recorded during a 10 s pre-CS period, during the 10 s CS–US interval and for 10 s following the termination of the US. Unit activity was evaluated separately for CS+ and CS− trials.

Following the last day of conditioning the animals were tested for the effects of (CDP) on baseline and conditioned unit responding. Testing was conducted in two 40-trial conditioning sessions carried out in the same day. In the first session the animal was injected with the vehicle (saline, 1 ml/kg) 15 min prior to the start of the session. Immediately after the end of the vehicle session animals were injected with CDP and 15 min later were given a second 40 trial conditioning session. A baseline session in which vehicle was injected prior to testing was run 48 h after the drug session to ensure that unit activity had returned to baseline. This sequence was run three times for three different doses of drug. The order of the doses of CDP administered was 10, 5, and 20 mg/kg i.p. At least 1 week was allowed to elapse between each dose in order to ensure clearance of the drug and its active metabolites.

2.6.2. Non-aversive context

The subjects for this condition were 14 male Sprague–Dawley rats. Animals were tested on three doses of CDP. Based upon a relatively flat dose–response curve for the doses selected for the aversive context, it was decided, for this group, to move the assessment of the dose–response function toward a lower dose. Therefore the three doses selected for this condition were 2, 5 and 10 mg/kg. For each dose animals were habituated in the recording chamber for 30 min. Baseline unit activity was then recorded for 15 min. Subsequently the animal was removed for the chamber and administered saline (1 ml/kg i.p.) and placed back in the chamber. Fifteen minutes after the saline injection, unit

activity was recorded for another 15 min. Finally, the animal was again removed from the chamber and injected with (CDP) in concentrations of either 2, 5 or 10 mg/kg. The animal was then replaced in the chamber and 15 min following CDP administration an additional 15 min of unit activity was recorded. Each animal received each dose of CDP, one per session. At least 1 week was allowed to elapse between doses. The order of doses for each animal was randomized.

As in experiment 1 units had a variable life span and did not necessarily last for the entire dosage regimen. If a unit was lost a search was made for another unit. If none was found the animal was terminated. For several of the animals the same unit was maintained for the entire dosage regimen.

2.7. Histology

Following the last recording session the animals were overdosed with pentobarbital and the brains were perfused with formalin. Frozen sections were taken at 40 μm. Electrode placements were verified by a photographic procedure adapted from Guzman-Flores, Alcarez, and Fernandez-Guardiola (Guzman-Flores et al., 1958).

3. Results

3.1. Conditioning data

Histological verification of the recording electrodes for animals in the conditioning experiment is shown in Fig. 1. Twenty animals which completed the sequence had electrodes in the lateral septal area. Two distinct groups of sites may be discerned, a lateral group with electrodes tips located in the dorsolateral/ventrolateral region of the septum (LSD/V) and a more medial group with tips in the intermediolateral septum (LSI). Eleven sites were in the LSD/V and nine in the LSI. Fig. 2 presents photographs of representative animals with electrodes in LSD (A) and LSI (B).

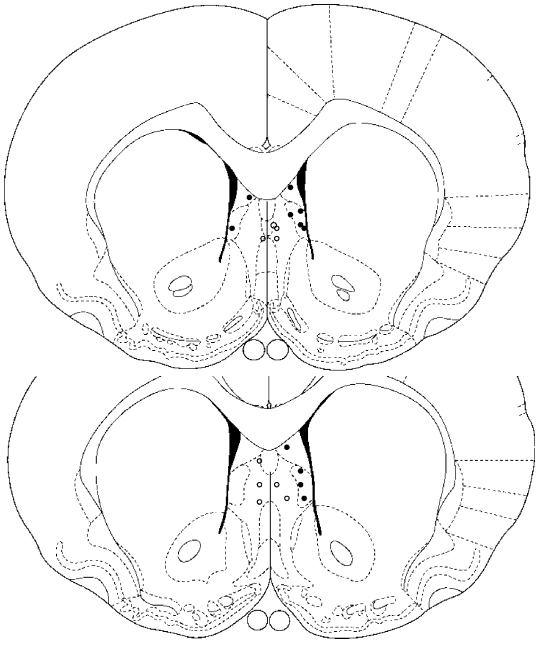

Fig. 1. Histological representation of electrode placements. ●=Dorsolateral/ventral group; ○=intermediate septal group. Plates adapted from Paxinos and Watson (1998).

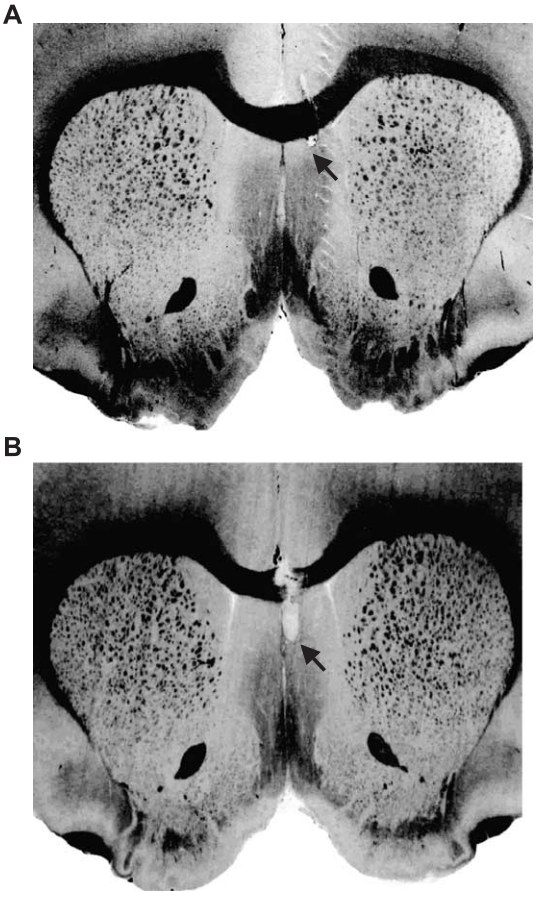

Fig. 2. Photomicrographs depicting representative placements of electrodes. (A) Dorsolateral septum. (B) Intermediolateral septum. Arrows point toward the electrode tips.

The determination of whether spikes seen on successive days of conditioning represent the same unit can be problematic. In general we classified a spike as coming from the same unit when it satisfied the following criteria (Thomas et al., 1991): (1) It was initially highly discriminable in amplitude and width from other spikes in the same electrode. (2) It changed only moderately over time and maintained its initial signal to noise ratio. (3) Its spontaneous rate was relatively constant over days. We assumed that a unit had changed when we observed a marked change in amplitude and waveform, a change in spontaneous rate, and in particular, a change in the electrode from which it was recorded.

As an example Fig. 3 shows 10 successive waveforms superimposed on each other on four successive days. Fig. 3A, B, and C were deemed to represent the same unit over three days. Fig. 3D represents a new unit subsequently

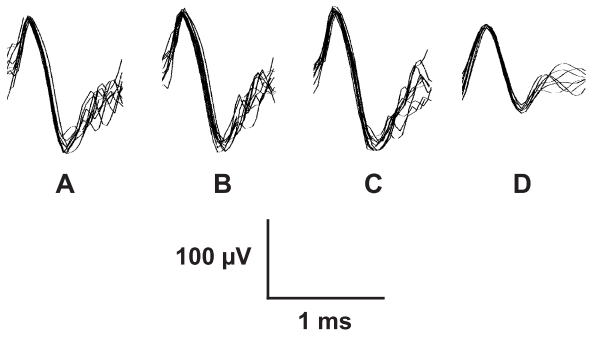

Fig. 3. Superimposed waveforms of spikes meeting the multiple criteria of a single unit over successive days. Each figure consists of 10 successive overlapping spikes. (A)–(C) depict a single unit maintained over three successive days. (D) depicts a new unit subsequently found in the same animal. The time bar represents 1 ms duration. The vertical bar represents 100 μV.

discovered in the same animal. Thus, although the same units were not necessarily maintained throughout the conditioning session it was still possible to derive a pseudo-learning curve for the groups of animals designated as LSD/V and LSI. For the derivation of this curve if a unit was lost during training another was substituted for it. Cellular activity is expressed as a unit activity ratio which compares the difference in the number of unit spikes between the CS and the Pre-CS period divided by the sum of the two (CS−PRE)/(CS+PRE). For each animal the unit activity ratio was determined for each trial and daily means calculated across the 20 CS+ and CS− trials of the session. This method allows for symmetrical depiction of excitation and inhibition around a zero point, with negative values reflecting suppression of activity during the CS compared with the pre-CS period and positive values reflecting facilitation during the CS. The scores vary from −1.00 (total suppression) to +1.00 (maximum increase in firing) with zero indicating no change. This derivation compensates to a large extent for differences in baseline rates of units and minimizes the effect of substitution of one unit for another. The learning curve actually contains data from twenty-six different units. The baseline rate for the cells ranged from 0.19 to 26.0 spikes/s with a median rate of 3.10 spikes/s.

Using this procedure it may be seen in Fig. 4A that there is a progressive decrease in the unit activity ratio for CS+ in the LSD/V group over sessions, and no change in unit activity to CS−. Cells in this septal region discriminate CS+ from CS− and in particular suppress activity to the conditioned fear stimulus. There were no consistent changes in unit activity to either CS in the LSI group.

These overall observations were supported by repeated measures analysis of variance (ANOVA) performed on the last conditioning session prior to the administration of drug, depicted in Fig. 4B. The ANOVA revealed an effect of histologic group [$F(1,14)=12.48$, $p<0.01$] and a CS effect [$F(1,14)=4.64$, $p<0.05$]. Fig. 5 depicts the results of a typical conditioning session for an animal in the LSD group, represented as peristimulus time histogram summed over the 20 trials. As can be seen unit activity is suppressed during CS+ compared to the Pre-CS period whereas no suppression is seen during CS−.

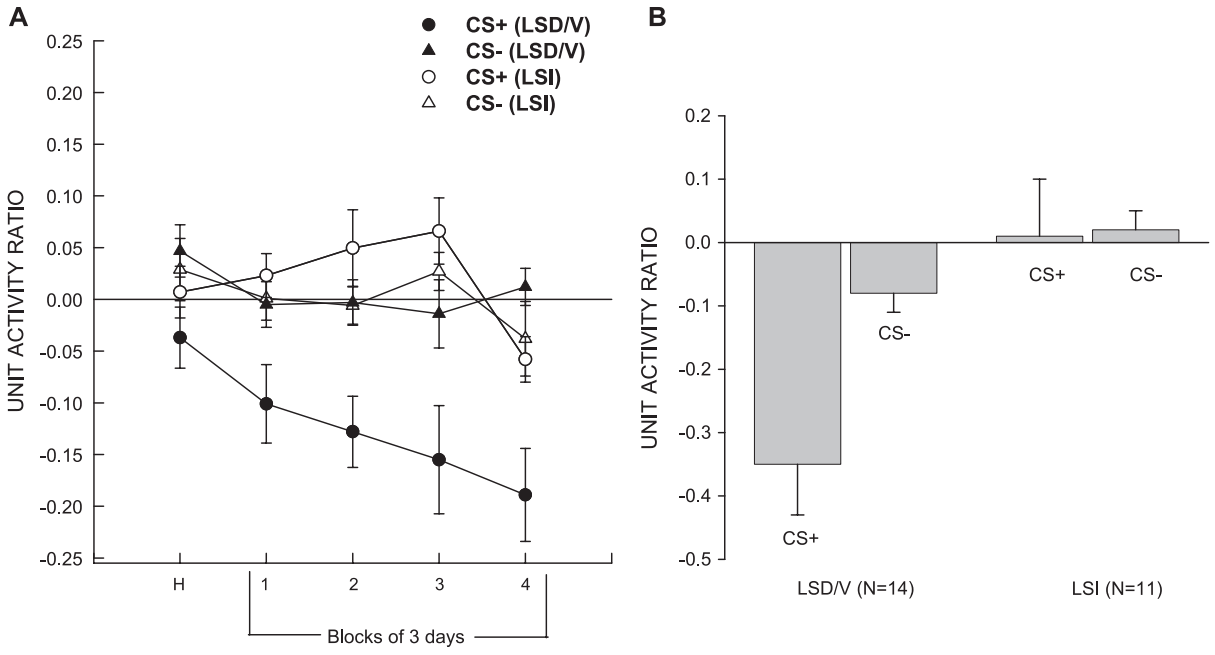

Fig. 4. Mean unit activity during Pavlovian conditioning for the two histological groups. H=habituation, one session only. LSD/V=dorsolateral/ventral group, LSI=intermediate septal group. (A) represents the course of conditioning over four blocks of three sessions. (B) represents the state of conditioning on the final session for which the statistical analysis was performed. The median baseline rate for group LSD/V was 2.91 spikes/s (range, 0.19–6.90 spikes/s). The median baseline rate for group LSI was 2.62 spikes/s (range, 0.79–4.44 spikes/s). The difference in baseline rates between the two groups was not statistically significant.

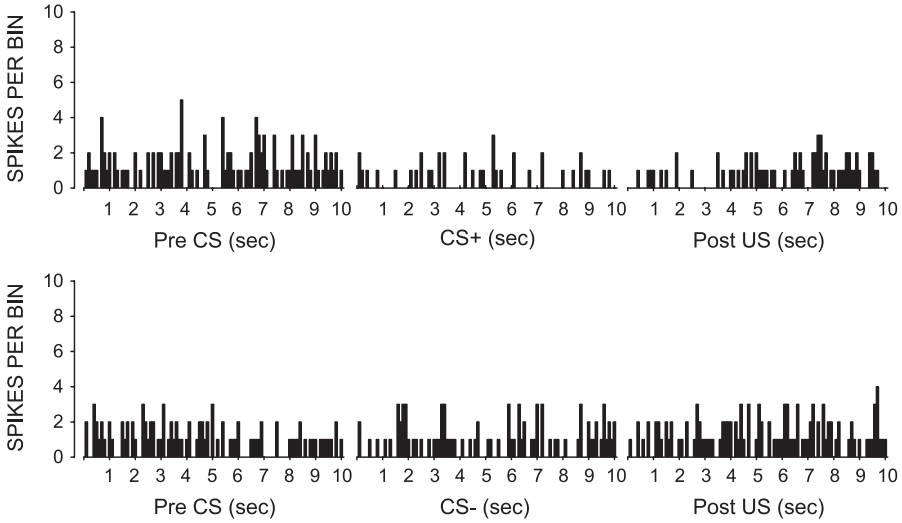

Fig. 5. Peristimulus time histograms for a unit in the dorsolateral septum. The data were taken from a subject on the final day of conditioning. The unit activity ratios for this subject were −0.23 and 0.06 for CS+ and CS−, respectively. The histograms are summed over 20 trials. Each of the Pre-Cs, CS and Post US are 10 s in duration and divided into 100 ms time bins. The overall lowering of unit responding throughout the 10 s of the CS+ is typical. There is little change in unit activity to CS− compared the equivalent pre-CS period.

3.2. Pharmacology

3.2.1. Aversive conditioning

The effect of CDP was examined on both spontaneous activity measured in the intertrial intervals and upon conditioned suppression of unit activity. Presumably the effect of the drug on baseline activity in the intertrial intervals provides a measure of the direct effect of the drug on unit activity in the septum in the context of the conditioning chamber. The effect of the drug was expressed as the mean percent change from the vehicle session to the drug session The results are depicted in Fig. 6. As may be seen in the figure, there was a substantial increase in unit activity to CDP in the LSD/V group and a substantial suppression of unit activity in the LSI group. This difference was supported by a significant groups effect in a repeated measures ANOVA [$F(1,15)=4.93$, $p<0.05$]. Interestingly, the dose response curve for the LSD/V group is flat, while there does appear to be a dose dependency for the LSI group. The reason for this may lie in the putative mechanism for the increase in unit firing in LSD/V to be discussed below.

We present here the effects of CDP on the conditioning of unit activity for the 10 mg/kg dose. As may be seen from Fig. 7, CDP blocks the conditioned suppression of unit activity in the LSD/V group. The drug had no effect

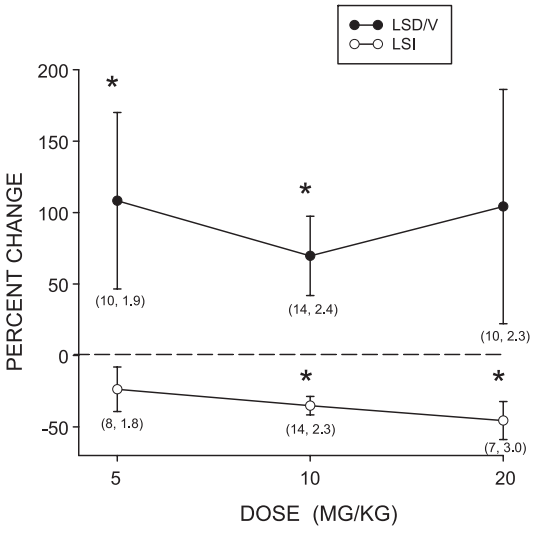

Fig. 6. Effect of CDP upon baseline unit activity in the two septal regions (LSD/V and LSI). CDP had a generally facilitatory effect upon baseline unit firing in LDS/V and a generally suppressing effect in LSI. The asterisk indicates a significant difference ($p<0.05$) between drug and control. The numbers in parentheses refer to the number of units recorded and the median baseline rate of firing (spikes/s).

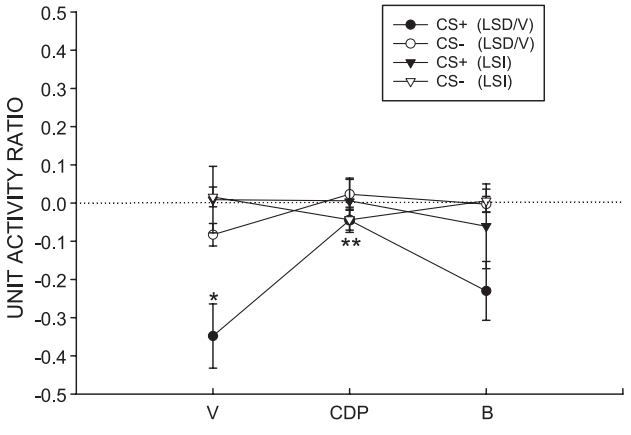

Fig. 7. Effect of CDP (10 mg/kg) upon conditioned unit activity in the two septal regions (LSD/V and LSI). Two sessions are compared, a session with vehicle (V) and a drug session (D). The asterisk indicates a significant difference ($p<0.01$) between CS+ and CS− in the vehicle session. Double asterisk indicates a significant difference ($p<0.01$) between the Vehicle and CDP session for group LSD/V for CS+. No other differences were significant. The effects of the other two doses were similar.

on CS− in this group nor on either CS for the LSI group. ANOVA shows a Groups effect [$F(1,14)=5.00$, $p<0.05$], Groups by CS interaction [$F(1,14)=5.99$, $p<0.05$], Drug effect [$F(1,14)=9.93$, $p<0.01$], and Groups by Drug interaction [$F(1,14)=18.38$, $p<0.001$]. The two other doses had similar effects upon the conditioned suppression of unit activity. The unit activity ratio for CS+ in the LSD/V group was −0.28 for the control and −0.03 for the 20 mg/kg group. A *t*-test revealed this difference to be significant $t(9)=2.81$, $p=0.025$. For the 5 mg/kg dose the unit activity ratio for CS+ was =0.14 and −0.08 for vehicle and drug, respectively. This difference did not reach statistical significance.

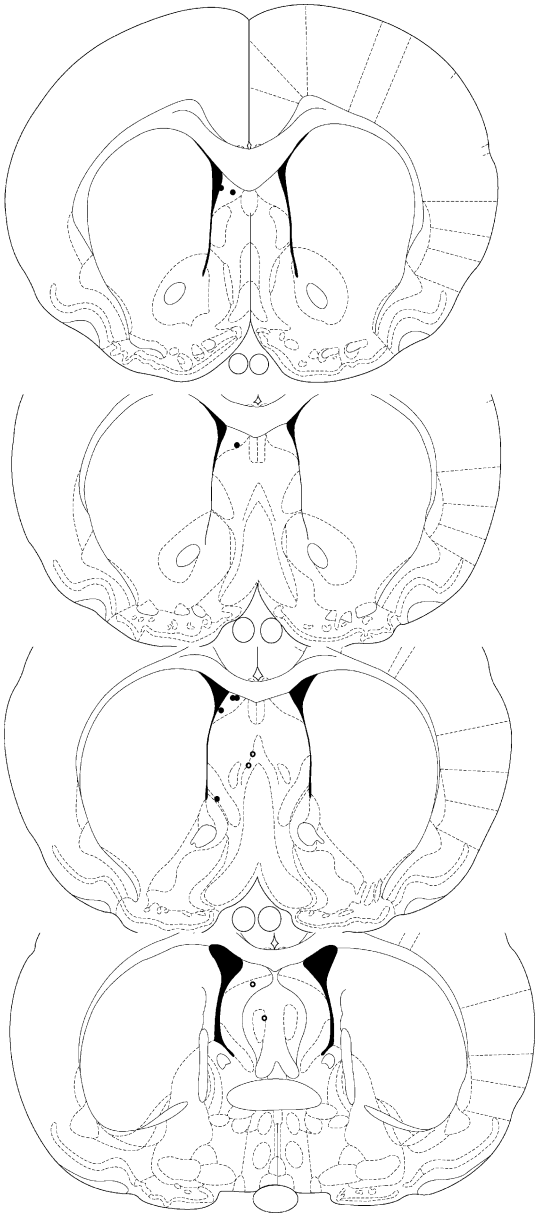

Fig. 8. Histological representation of electrode placements for animals in the non-aversive context. ●=Dorsolateral/ventral group; ○=intermediate septal group. Plates adapted from Paxinos and Watson (1998).

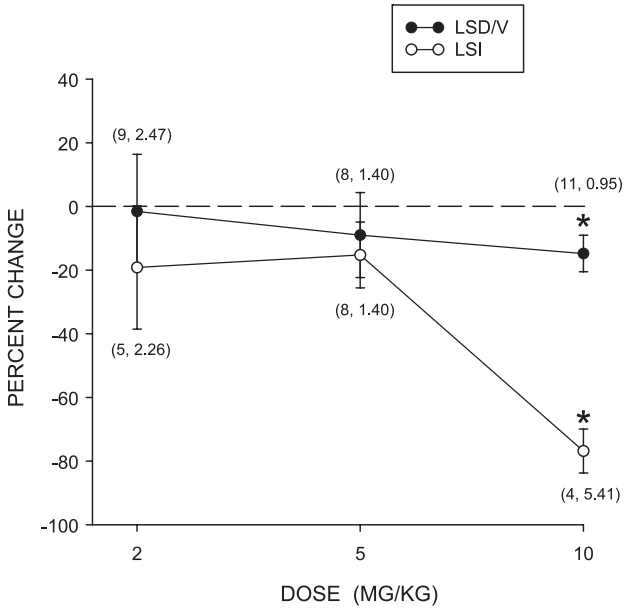

Fig. 9. Effect of CDP upon spontaneous unit activity in the two septal regions (LSD/V and LSI). In the non-aversive context the effect of CDP upon unit activity was minimal except at the highest dose. The asterisk indicates a significant difference ($p<0.05$) between drug and control.

3.2.2. Non-aversive context

Fig. 8 shows the placement of the electrodes in the animals that completed the experiment. The electrode tips were distributed in the regions LSD/V and LSI. Fig. 9 presents a plot of the percent change in unit activity in the CDP session compared to the immediately preceding saline session for each dose of CDP. As may be seen in the figure CDP had no facilitatory effect upon unit activity in either region of the septum. A one-sample *t*-test comparing the percent change to zero yielded significance only at the 10 mg/kg dose $t(10)=2.57$, $p=0.03$. As may be seen the effect of CDP at that dose was to suppress unit activity in LSD/V. A similar trend is apparent for the effect of CDP in LSI. Again the only significant effect was at the 10 mg/kg dose $t(3)=11.14$, $p=0.002$.

4. Discussion

The principal finding of the present study was that cells in the lateral septum, which are inhibited by aversive conditioned stimuli in the awake freely moving animal, increase their rate of firing in response to the benzodiazepine anxiolytic chlordiazepoxide. In general the results of this study confirm the results of our previous research (Thomas et al., 1991) that cells in the more lateral regions of the septum respond to the hedonic properties of conditioned stimuli. The effect of Pavlovian aversive conditioning was to decrease unit activity to CS+ in the dorsolateral and ventrolateral septal nuclei but not in the more medial intermediate nucleus.

The recording of the response of single neurons to pharmacological agents is one of a variety of methods for determining the mechanism of therapeutic action of drugs. Such recording in the awake freely moving animal has the significant advantage of being able to relate such responses directly to behavior. In this case the ability to record unit activity in the context of aversive conditioning can supply important information relating BZDs, unit activity, and fear. Thus, the benzodiazepine CDP reduced the conditioned suppression of unit activity in the lateral cell groups. Such a reduction is highly consistent with its anxiolytic properties and parallels its effect on behavior suppressed by aversive conditioning (Cook and Davidson, 1978).

Equally germane to the putative mechanism of action of CDP is the finding that in the aversive context CDP increased spontaneous unit activity of these cells in the baseline period in the absence of the CS. In contrast CDP did not increase spontaneous unit activity in a non-aversive context. The literature indicates that in aversive conditioning not only does the conditioned cue elicit fear, but the context does as well (Fanselow, 2000; Phillips and LeDoux, 1992; Sparks and LeDoux, 1995). It seems likely then, that in the aversive paradigm some degree of contextual conditioning took place, exerting a tonic inhibitory effect on unit firing in the experimental environment. This is in agreement with research that shows that the septum plays a substantial role in contextual conditioning (Sparks and LeDoux, 1995).

The absence of an increase in unit activity in response to the administration of CDP in the non-aversive context is in striking contrast to the effect of CDP in the aversive context. It appears that in the absence of a fear stimulus CDP has little effect at doses which in the aversive context increase unit activity in LSD/V. These data are consistent with those of Givens and Breese (Givens and Breese, 1990a,b) who found that ethanol, which binds to the BZD-GABA-Cl^- complex, has little effect on neurons in LS in urethane anesthetized as well as unanesthetized rats. On the basis of our results it might be expected that ethanol, which has anxiolytic properties akin to CDP, would elevate septal firing in an aversive context.

If, as we have proposed, the lateral septum is involved in the relief of fear, then the increased unit activity in the lateral septum to CDP in the aversive context would suggest that benzodiazepines might exert their anxiolytic effect by activating neurons in the lateral septum and related regions that have been inhibited by fear. The mechanism whereby BZDs might increase cellular activity in the lateral septum remains at this time a matter of speculation. The BZDs work primarily via the BZD-GABA-Cl^- complex and therefore would be expected to have a generally inhibitory action on cellular activity. This has typically been the case in most structures tested including the hippocampus (Chou and Wang, 1977; Steffensen and Henriksen, 1992), amygdala (Chou and Wang, 1977), dentate gyrus (Steffensen and Henriksen, 1992), substantia nigra (Ross et al., 1982), locus coeruleus (Grant et al., 1980), and in the case of the present experiment, in intermediolateral septum.

It should be noted, however, that electron micrographs reveal GABA–GABA synapses in interneurons in the lateral septum (Onteniente et al., 1987). The synapsing of GABAergic boutons onto other GABAergic neurons is important because this connection allows for a possible disinhibitory mechanism within the septum following increased GABAergic activity. Thus, BZDs may have a facilitatory effect on lateral septal neurons by means of a disinhibition mechanism. It may be that the complexity of GABAergic connections within the lateral septum accounts for the lack of a simple dose response effect of CDP in our experiment. It is of interest that the anxiolytic effect of direct application of BZDs or GABA agonists into the lateral septum also does not show a simple dose response effect (Drugan et al., 1986; Grishkat, 1991).

BZDs, in addition to their anxiolytic effect, have been shown to have substantial effects upon several forms learning and memory. These include spatial tasks such as the Morris water maze (McNaughton and Morris, 1987) and non-spatial working memory tasks (Olaman and McNaughton, 2001). These tasks are particularly sensitive to hippocampal damage and very likely reflect the effect of BZDs on hippocampal functioning. Pavlovian fear conditioning also appears to be impaired in a variety of situations. However, the effects of BZDs on fear conditioning have been variable and context dependent (Harris and Westbrook, 2001). To the extent that there is an impairment of fear conditioning this likely represents a diminution of amygdala function (Davis et al., 1994). A possibility that bears further research is that the lateral septum may have an effect on the expression and learning of fear by modulating activity in the amygdala.

The lateral septum is strategically situated to serve as a link between the hippocampus and the brainstem emotional circuits as well between the hippocampus and the amygdala (Sheehan et al., 2004; Thomas, 1988). It receives a substantial input from the hippocampus (Risold and Swanson, 1997). The lateral septum projects to the central nucleus of the amygdala either directly (Volz et al., 1990) or via the bed nucleus of the stria terminalis (Jakab and Leranth, 1995). There is evidence that the behavioral effects of the septum are mediated by the amygdala, especially the central nucleus (Grishkat, 1991; Melia et al., 1992). Recent research in our laboratory (Thomas and Sancar, 2001) has shown that activation of the lateral septum by electrical stimulation has a profound inhibitory effect upon cells in the central nucleus of the amygdala likely mediated by $GABA_A$ receptors.

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