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Role of the Dorsomedial Hypothalamus in Mediating the Response to Benzodiazepines on Trial 2 in the Elevated Plus-Maze Test of Anxiety

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Trial 2 in the elevated plus-maze provides an animal model of specific phobia (fear of heights). On this trial, rats no longer respond to benzodiazepines. The present experiment examined the role of the dorsomedial hypothalamus in mediating insensitivity to chlordiazepoxide on trial 2. Rats received a 5 min exposure to the maze, undrugged. Forty-eight hours later, rats injected with control infusions into the dorsomedial hypothalamus showed the usual lack of response to chlordiazepoxide (5 mg/kg, i.p.). However, those receiving lidocaine injections (40 μg/μl in a volume of 0.2 μl) in the dorsomedial hypothalamus (producing functional inactivation), immediately before trial 2, responded with an anxiolytic response to chlordiazepoxide, characterised by an increased percentage of time on the open arms and by an increased number of entries into, and time spent on, the distal portions of the open arms. Since the lidocaine injections were without anxiolytic effects, our results suggest that this region of the hypothalamus regulates the functional state of benzodiazepine receptors in other brain regions. ***[Neuropsychopharmacology 21:312–320, 1999]*** *© 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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The benzodiazepines provide an effective treatment for generalised anxiety disorder, but they are less effective in other anxiety disorders and are not at all effective against simple or specific phobias, for which exposure therapy is the best treatment (Marks 1987; Tyrer 1989). Simple phobias are chronic illnesses of moderate severity, often co-morbid with other anxiety disorders and which are seldom well treated (Goisman et al. 1998).

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There are several different animal tests of anxiety and the challenge is to develop animal tests that best reflect the differing anxiety disorders that are recognised clinically. Principal component analysis has confirmed that the measures derived from different animal tests do, indeed, load on independent factors and are thus reflecting quite distinct states of anxiety (File 1991). There is, also, growing evidence that different brain regions and neurotransmitter systems modulate different measures of fear and anxiety (Pesold and Treit 1994, 1995; File and Gonzalez 1996; Gonzalez and File 1997; Killcross et al. 1997; Treit and Menard 1997), and neuroimaging studies have shown different patterns of brain activation in different anxiety disorders (Lucey et al. 1997; Birbaumer et al. 1998; Fredrikson et al. 1995; Wik et al. 1993).

The elevated plus-maze is a well validated (Pellow et al. 1985) and widely used test of anxiety in which a rat

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or mouse is faced with a choice of open elevated arms or those enclosed by a high wall. Increases in the percentage of time spent on the open arms and open arm entries indicate an anxiolytic effect, such as caused by benzodiazepines. Surprisingly, rats and mice do not show habituation to anxiety (File 1990; File and Zangrossi 1993; File and Gonzalez 1996; Gonzalez and File 1997; Rodgers et al. 1992; Rodgers and Shepherd 1993) or to the elevation in corticosterone (File et al. 1994; Holmes et al. 1998). Treit et al. (1993) have found that on the first exposure to the plus-maze it is the open aspect of the arm, rather than its elevation, that is the main anxiogenic stimulus. In animals exposed to the maze for the first time, benzodiazepines reliably increase the percentage of time spent on the open arms and the percentage of entries onto open arms. However, it soon became clear that if rats or mice were replaced on the plus-maze for a second 5-minute trial, they were insensitive to the anxiolytic effects of benzodiazepines or barbiturates (File 1990; File 1993; Rodgers et al. 1992; Rodgers and Shepherd 1993). The drug state of the animal on trial 1 is unimportant to this insensitivity to benzodiazepines on trial 2 and the interval between trials can be from 24 hr to 2 weeks; the crucial factor is experience of the open arms (File et al. 1990) which must include exploration of, and head-dipping over, the edges of the arm and hence knowledge of the drop (Fernandes and File 1996). If experience of the drop is prevented on trial 1 by placing small edges around the open arms, then rats will respond to benzodiazepines on trial 2 (Fernandes and File 1996). Thus, whilst the state of fear or anxiety generated by initial exposure to the plus-maze is unconditioned, by trial 2 it was replaced by a different form of fear which was rapidly acquired during the trial 1 experience. The acquisition of this different form of fear, based on experience of the drop, is probably based more on tactile than visual cues. This is because the rat is myopic and with an undifferentiated floor would be unable to make graded depth judgments; it is also likely that depth is judged in an "all-or-none" manner—low enough and safe to jump or high enough to evoke fear. The lack of sensitivity to benzodiazepines on trial 2 is not because the animals have habituated to the apparatus. There is no habituation to the corticosterone response (File et al. 1994; Holmes et al. 1998) and the behavioural measures are either unchanged (Pellow et al. 1985; File 1990; Taukulis and McKay 1992) or show further reductions in time spent on the open arms, indicating increased anxiety (Rodgers et al. 1992; Rodgers and Shepherd 1993; Treit et al. 1993; Fernandes and File 1996; File et al. 1998). Because of the insensitivity to benzodiazepines on trial 2, and the importance of the fear of heights on this trial, it was proposed that the nature of anxiety evoked was similar to a simple, or specific, phobia (File and Zangrossi 1993; File et al. 1996). Fear of heights is the most common of the simple phobias (Goisman et al. 1998). Principal component analyses confirmed that the measures of anxiety for trials 1 and 2 depend on two independent factors (File et al. 1993; Rodgers and Johnson 1995; Fernandes and File 1996) and thus reflect two different states of anxiety.

Previously, we have shown that rats that received lidocaine injections into the basolateral amygdala (thus inducing a temporary functional deactivation), immediately after trial 1 in the plus-maze, responded with an anxiolytic response to chlordiazepoxide when tested 48 hr later on trial 2 (File et al. 1998). Those that received a sham lesion after trial 1 showed the usual lack of response to chlordiazepoxide on trial 2. Thus the basolateral amygdala plays a crucial role in the consolidation of information that leads to the formation of a different type of fear and subsequent insensitivity to benzodiazepines on trial 2.

However, from a clinical point of view, it is perhaps more important to identify the brain areas and neurotransmitters that are crucial to the expression of this fear and, therefore, the present experiment examined the role of the dorsomedial hypothalamus by functionally inactivating it (by lidocaine injection) just prior to trial 2 in the plus-maze. Localised injection of lidocaine into the brain produces a temporary functional block of neural activity (Salinas et al. 1993; Packard and McGaugh 1996). The dorsomedial nucleus of the hypothalamus receives input from the amygdala (Bernardis and Bellinger 1987; LeDoux et al. 1988), the raphé nuclei and the locus coeruleus (Swanson 1987), and has been postulated, together with the amygdala and the dorsal midbrain central grey, to form part of an integrated neural circuit responsible for the expression of aversive states (Graeff 1981; Panksepp 1990). The role of the dorsomedial hypothalamus has not been explored on trial 2 in the plus-maze, but ibotenic lesions of this nucleus resulted in an anxiolytic effect on trial 1 (Inglefield et al. 1994). In rats tested up to four times in the plus-maze, infusions of bicuculline and muscimol into this area had anxiogenic and anxiolytic effects, respectively (Shekhar 1993).

Because only one dose of chlordiazepoxide was to be used on trial 2, an initial group of rats was used to confirm an effective anxiolytic dose on trial 1 in the plus-maze. These rats were not used in the subsequent experiment. This precaution was taken because of seasonal and batch differences in sensitivity to the anxiolytic effects of benzodiazepines (File and Hitchcott 1990).

MATERIALS AND METHODS

Animals and Surgery

Male hooded Lister rats (Harlan, Bicester, UK), weighing 270–320 g, were individually housed after surgery in a dimly lit room maintained at 22°C, with lights on

from 0700–1900 hr. Food and water were freely available. Before surgery, the stereotaxic coordinates were verified histologically on four rats from the same batch. One week after arrival, animals were anaesthetised by inhalation of 3% halothane (May and Baker, U.K.) in oxygen and positioned in a stereotaxic frame (Kopf Instruments, California), such that bregma and lambda were in the same horizontal plane. Three indentations were made in the skull using a dental drill (Casali, Milan, Italy) to accommodate brass screws which, together with the application of dental cement, held the cannula in place. Stainless steel guide cannulae 12 mm long (23 gauge; Cooper's Needle Works Ltd, Birmingham, UK) were positioned at 2.4 mm posterior to bregma, ±2.7 mm lateral and −7.0 mm vertical from the skull at an angle of 19° towards the midline, thus siting them 2 mm above the target area. Cannulae were kept patent using 12 mm long stainless steel stylets (30 gauge; Cooper's Needle Works Ltd, Birmingham, U.K.) and these were changed daily. The animals were allowed at least five days recovery from surgery before behavioural testing. These experiments were conducted in compliance with the U.K. Animals (Scientific Procedures) Act 1986 (Project Licence 70/4041).

Drugs and Microinfusion

Lidocaine (2-Diethylamino-N-[2,6-dimethylphenyl]-acetamide hydrochloride; Sigma Chemical Co., Poole, Dorset, U.K.) was dissolved in artificial cerebrospinal fluid (aCSF) of the following composition (mM); NaCl 126.6, $NaHCO_3$ 27.4, KCl 2.4, KH_2PO_4 0.5, $CaCl_2$ 0.89, $MgCl_2$ 0.8, Na_2HPO_4 0.48 and glucose 7.1, p.H. = 7.4. Five min prior to trial 2, each rat received bilateral infusions of either lidocaine (40 μg/μl) or aCSF, delivered at the rate of 0.2 μl/min for 1 min, using a microdialysis pump (CMA/102; Biotech Instruments, Stockholm, Sweden). The injection needle (30 gauge) was inserted 2 mm below the tip of the cannulae and was left in place for one additonal minute to allow diffusion away from the tip. Chlordiazepoxide hydrochloride (Sigma) was dissolved in distilled water to a concentration of 2.5 mg/ml. On trial 2, rats received an intraperitoneal injection of chlordiazepoxide (5 mg/kg, i.p.) or an equal volume of distilled water, 30 min before testing.

Apparatus

The plus-maze was made of wood and consisted of two opposite open arms 50 × 10 cm, and two opposite arms enclosed by 40 cm high walls. The arms were connected by a central 10 × 10 cm square, and thus the maze formed a "plus" shape. The maze was elevated 50 cm from the floor and lit by dim light. A closed circuit TV camera was mounted vertically over the maze and the behaviour was scored from a monitor in an adjacent room. The numbers of entries onto open and enclosed arms and the times spent on the open and closed arms and on the central square were recorded. An arm entry was defined as being when all 4 paws entered the arm; exit from an arm was defined as being when the forepaws left that arm. The scores were entered directly into an IBM computer. The percentage of open arm entries and that of time spent on the open arms provide the measures of anxiety. The number of closed arm entries is the best measure of general activity (File 1991). In addition, the number of entries into the distal portions of the open arms (the last half of the open arms) and the time spent on the distal portions were recorded. These measures depend on the same anxiety factor as the percentage of open arm entries and that of time spent on the open arms (Fernandes and File 1996), but provide a better measure of the rat's willingness to remain exposed to the drop once an open arm has been entered.

Procedure

On the first test day, each rat was placed in the central square of the plus-maze and given a 5 min undrugged trial in the maze. Rats were then randomly allocated, half to receive chlordiazepoxide injections (5 mg/kg, i.p.) 30 min before trial 2 and half to receive control (distilled water, i.p.) injections. Each of these groups was then subdivided so that half received intra-hypothalamic injections of lidocaine and half received aCSF. After verification of cannula placements there were 8–9 rats in each group. Trial 2 took place 48 hr after trial 1 and the rats were tested in an order randomised for drug treatment. Rats were scored for 5 min, by an observer who was blind to their drug treatment. Testing on both days took place between 0800 and 1200 hr and the maze was thoroughly cleaned after each rat was removed.

Histology

At the end of behavioural testing, all animals were sacrificed, the brains removed, and the injection site verified histologically [according to the atlas of Paxinos and Watson (1986)], by a person blind to drug treatment. Frozen brains were sectioned on a Kryomat (Leitz Wetzler, 31970) and 20 μm sections were taken until the site of the needle tip could be seen. Placements between 2.0 and 3.5 mm posterior to bregma, ±0.2 to 0.8 mm lateral and 8.4 to 9.0 mm vertical from the skull were considered as falling into the target area. Data from rats with placements falling outside the target area were not included in the statistical analysis.

Statistics

As the measures derived from the plus-maze were normally distributed and there was homogeneity of vari-

ance between the groups, the data were analysed by a two-way analysis of variance with central injection and intraperitoneal drug treatment on trial 2 as the two factors. After ANOVA, comparisons between individual groups were made with Duncan's tests. The significances of these individual groups are shown in the figures. The between-trial changes in the scores of the undrugged rats were analysed with a two-way split-plot ANOVA, with trials as the repeated measure and central injections as the independent factor.

RESULTS

The target area for our injections into the dorsomedial hypothalamus is shown as shaded in Figure 1. Also,

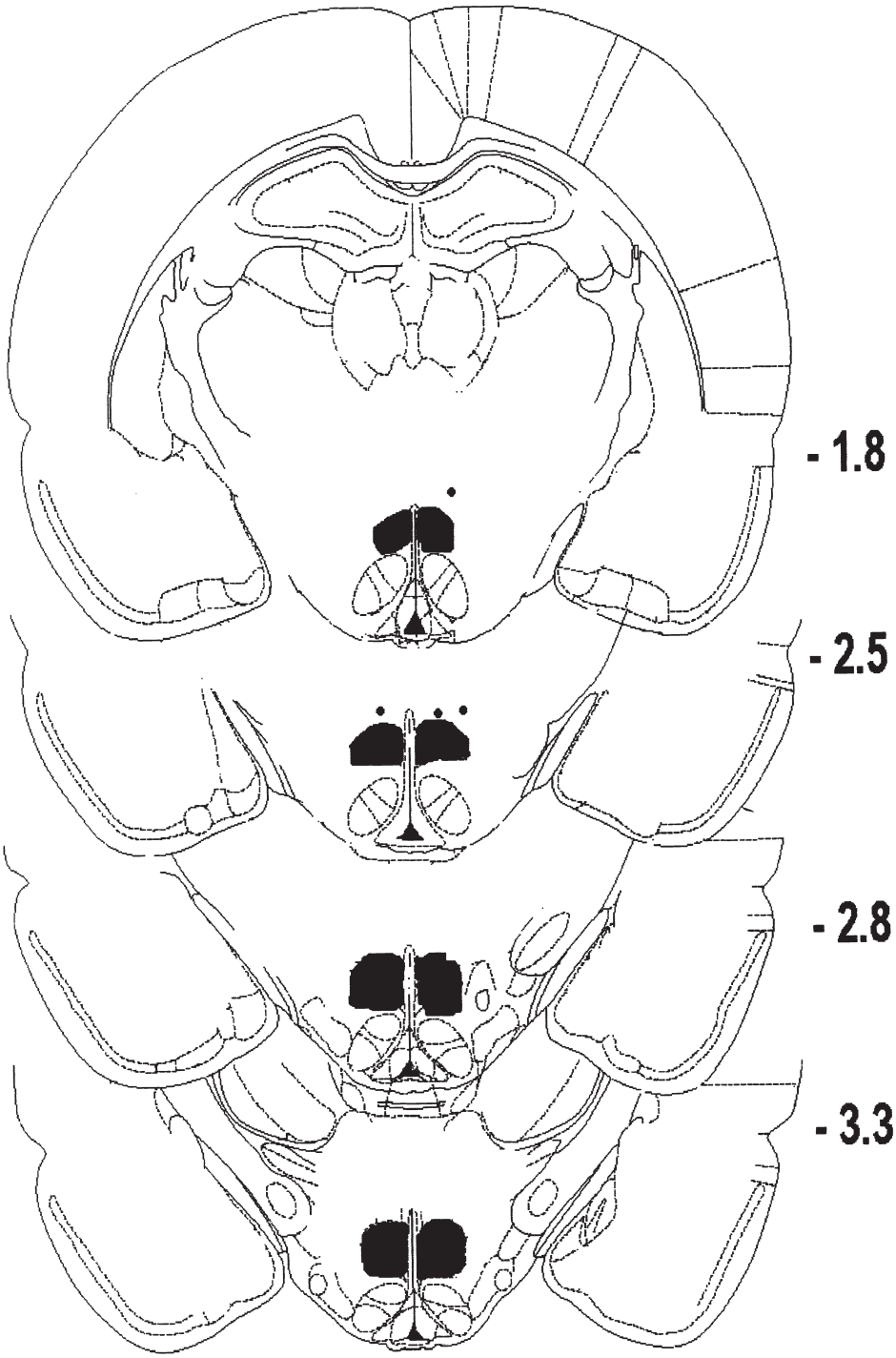

Figure 1. Diagrammatic representation of coronal sections from 1.8 to 3.3 mm posterior to bregma showing the target area (shaded) of the dorsomedial hypothalamus. Placements falling outside the target area are shown by filled circles marking the tip of the injection needle.

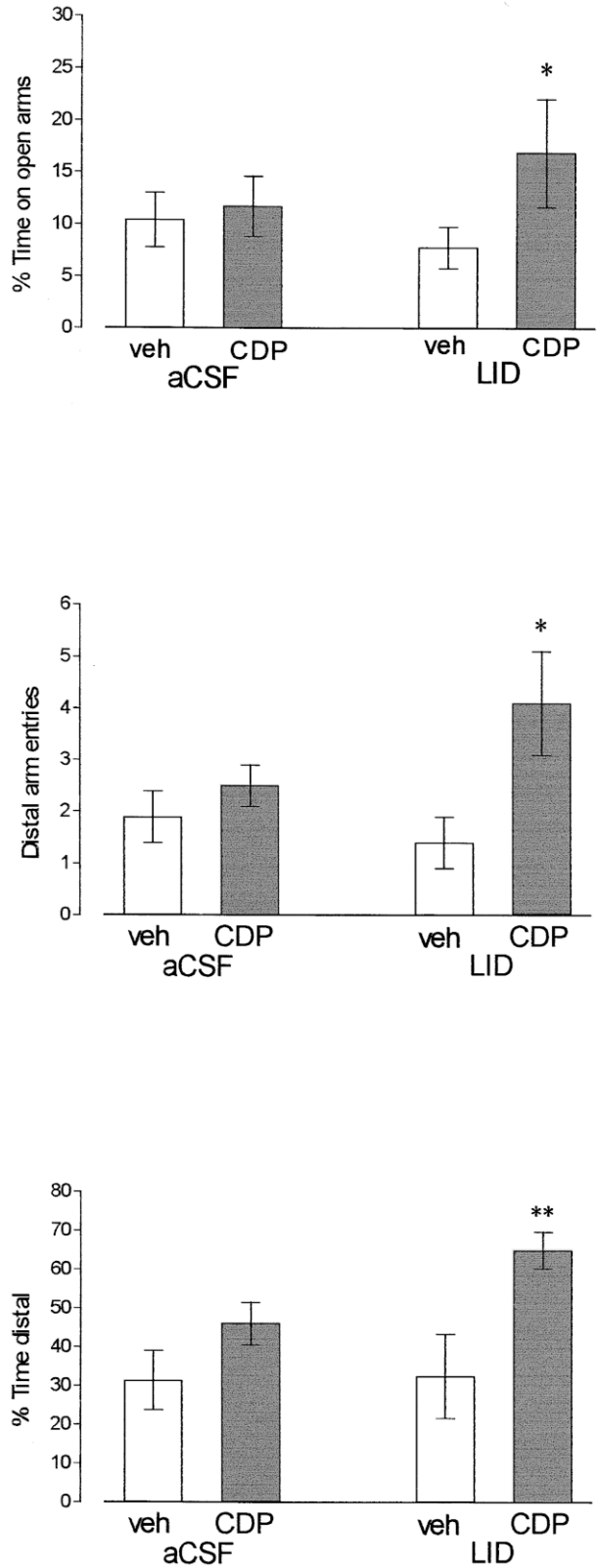

Figure 2. Mean (± s.e.m.) percentage of time spent on the open arms and percentage of entries into and time spent on the distal portion of the open arms on TRIAL 2 in the plus-maze by rats tested 30 min after intraperitoneal injection with distilled water (veh) or chlordiazepoxide (CDP, 5 mg/kg) and 5 min after injection into the dorsomedial nucleus of the hypothalamus of artificial CSF (aCSF) or lidocaine (LID). $^{**}p < .01$, $^{*}p < .05$ compared with veh group, Duncan's test after ANOVA

shown by filled circles, are the injection sites that fell outside this area. In all cases, the placement error was unilateral; in one case, the animal was in the lidocaine-CDP group, two cases were in the aCSF-CDP group, and in one case, the animal was in the aCSF-vehicle group. The scores for these animals were excluded from the statistical analyses, but there were too few cases to provide an analysis of the effects of injections outside the target area.

Figure 2 shows that rats which received the aCSF injection prior to trial 2 showed the usual insensitivity to chlordiazepoxide on this trial, whereas those which received the lidocaine injection into the dorsomedial hypothalamus responded to chlordiazepoxide with significant increases in the percentage of time spent on the open arms, the number of entries into the distal portion of the arms, and the percentage of time spent in the distal portion ($F(1,29) = 3.9$, 5.8 and 9.9 respectively; $p < .05$ in all cases).

Chlordiazepoxide was without effect in either group in the number of closed arm entries, the time spent in the central square, or the percentage of open arm entries ($F(1,29) < 1.0$, in all cases) (Table 1).

It can be seen, from the data presented in Figure 2 and Table 2, that the lidocaine injection itself was without effect on any of the measures in the plus-maze ($F(1,29) = 1.8$ for percentage of time on the distal portion of the open arms; $F < 1.0$ for all other measures).

Table 2 shows the scores for the rats tested undrugged on both trials; the subgroups did not differ on their trial 1 performance. These rats showed significant decreases in the two measures of anxiety, the percentage of time spent on the open arms and that of open arm entries ($p < .01$, in both cases), but no change in the measure of locomotor activity and the number of closed arm entries. It also shows an identical pattern for the rats with the lidocaine lesion of the dorsomedial hypothalamus, thus showing that this lesion did not disrupt the between-trial changes in behaviour.

DISCUSSION

The animals that received the aCSF injections showed the typical pattern of behaviour on trial 2, i.e., did not respond to chlordiazepoxide with an increase in the percentage of open arm entries or with an increase in the percentage of time spent on the open arms. This

Table 1. Mean (± SEM) Percentage of Entries Into Open Arms, Number of Closed Arm Entries, and Time (s) Spent in the Central Square by Rats Tested on Trial 2 in the Elevated Plus-Maze 30 Min after i.p. Injection with Vehicle or Chlordiazepoxide (5 mg/kg, CDP) and 5 Min after Dorsomedial Hypothalamic Infusion of Artifical CSF (aCSF) or Lidocaine

Hypothalamic infusion	aCSF	aCSF	Lidocaine	Lidocaine
i.p. injection	Vehicle	CDP	Vehicle	CDP
Time (s) in centre	71.9 ± 10.3	52.0 ± 10.0	50.1 ± 7.4	66.1 ± 9.7
Closed arm entries	13.4 ± 1.4	11.3 ± 1.3	12.5 ± 1.1	13.7 ± 0.8
% open arm entries	19.6 ± 2.9	20.7 ± 2.8	19.5 ± 3.2	22.2 ± 3.6

lack of sensitivity to chlordiazepoxide is not due to a change in baseline, since the baseline of the controls was considerably lower (indicating higher anxiety) on trial 2 than on trial 1 and it is easier to show an anxiolytic response from a lower baseline. The change in baseline from trial 1 to trial 2 in measures of anxiety has been seen frequently in other experiments and may indicate that the state of anxiety generated on trial 2 is greater than that generated on trial 1. There is certainly no habituation to anxiety or to the corticosterone stress response (File et al. 1994; Holmes et al. 1998). The lack of response to the test dose of chlordiazepoxide is also not due to locomotor habituation, as there was no change in the number of closed arm entries from trial 1 to trial 2. Numerous experiments have found insensitivity to the anxiolytic effects of chlordiazepoxide on trial 2, without any locomotor habituation (e.g., Gonzalez and File 1997; File et al. 1998).

The strategy employed in this experiment was designed to determine whether inactivation of the dorsomedial nucleus of the hypothalamus would re-instate a response to chlordiazepoxide on trial 2. This seemed to have happened with some, but not all, aspects of behaviour. Thus, the lesioned rats showed an anxiolytic response to chlordiazepoxide, as measured by an increased percentage of time spent on the open arms, but did not show an increase in the percentage of open arm entries. This suggests that the lesion did not change the avoidance component of the response, but that once an open arm had been entered, the escape component of the response was changed. This interpretation is further strengthened by the finding that the lesion also modified the effects of chlordiazepoxide on entries into the distal portions of the open arms and the time the rats spent there. Graeff et al. (1996) have previously reported differential regulation by the dorsal raphé 5-HT system of these two aspects of behaviour in the open arm, and the dorsomedial hypothalamus is an area that has been particularly associated with mediating escape behaviours (Fuchs and Siegel 1984; Brandao et al. 1986; Milani and Graeff 1987).

Phobic disorders are characterised by avoidance of the feared object or situation and, when confronted with it, intense fear and escape responses. To the extent to which trial 2 in the plus-maze can be considered as an animal model of a specific phobia, our results suggest that the dorsomedial hypothalamus plays a strong role in the escape response, but has little effect on the avoidance component. It would be important to determine whether inactivation of the dorsomedial hypothalamus affected the corticosterone stress response elicited on trial 2. Separation of the different components of a response to an aversive situation has also been found for the basolateral nucleus of the amygdala, which seems to control the avoidance component, but not response suppression (File et al. 1998; Killcross et al. 1997). If the anatomical separation of different components of a response to an aversive situation is also mir-

Table 2. Mean (± SEM) Percentage of Time Spent on the Open Arms, Percentage of Entries Into Open Arms, and Number of Closed Arm Entries Made by Rats Tested Undrugged on Both Trials 1 and 2, but after Dorsomedial Hypothalamic Infusion of Artifical CSF (aCSF) or Lidocaine 5 min Before Trial 2

Hypothalamic Infusion	aCSF	aCSF	Lidocaine	Lidocaine
Trial	Trial 1	Trial 2	Trial 1	Trial 2
% time open arm	24.7 ± 2.3	10.4 ± 2.6[a]	28.1 ± 2.7	8.3 ± 2.6[a]
% open arm entries	27.1 ± 1.3	19.6 ± 2.9[a]	32.9 ± 2.5	19.5 ± 3.2[a]
Closed arm entries	13.2 ± 1.1	13.4 ± 1.4	12.8 ± 1.3	12.5 ± 1.1

[a] $p < .01$ compared with trial 1.

rored by a pharmacological distinction, this could at least partly explain why drug treatment of specific phobias has not been very successful.

The most striking feature of behaviour on trial 2 in the plus-maze is its insensitivity to benzodiazepines after systemic or central administration (File 1990, 1993; Rodgers et al. 1992; Rodgers and Shepherd 1993; Gonzalez and File 1997; Gonzalez et al. 1998). Inactivation of the dorsomedial hypothalamus changed this pattern and the rats were able to respond to chlordiazepoxide. However, the lidocaine infusion itself did not have an anxiolytic effect and, furthermore, the lidocaine infused rats showed the same between-trial changes in scores as the control animals. This contrasts with the results reported for trial 1 in the plus-maze, following ibotenic lesions of the dorsomedial hypothalamus. Inglefield et al. (1994) reported increased percentage of open arm entries, percentage of time on the open arms, and number of closed arm entries. This last measure indicates increased locomotor activity in the plus-maze which was also found in another test situation. Therefore, the lesion may not have had a specific anxiolytic effect, but the results certainly indicate an overall disinhibition. In another test of anxiety, the social interaction test, Inglefield et al. (1994) found no effect of the lesion, but Shekhar and Katner (1995) found that injection of GABA agonists and antagonists into this region did have anxiolytic and anxiogenic effects, respectively. Overall, the results suggest that the dorsomedial hypothalamus plays an important role in the behavioural as well as the physiological response (Shekhar et al. 1993; Keim and Shekhar 1996) to aversive situations; the precise nature of this role needs further investigation.

The fact that the lesion itself had no anxiolytic effect, but re-instated the anxiolytic response to chlordiazepoxide, indicates that it is the responsiveness of benzodiazepine receptors outside this brain region that was changed. PET studies have shown reduced relative cerebral blood flow in prefrontal, orbitofrontal, temporopolar, and posterior cingulate cortex of snake and spider phobics when exposed to pictures of their phobic objects (Wik et al. 1993; Fredrikson et al. 1995). Kellogg et al. (1993) have previously shown the importance of hypothalamic mediation of the response of cortical GABA-benzodiazepine receptors to environmental stressors. They showed that lesions of the paraventricular nucleus of the hypothalamus altered stressor-induced changes in benzodiazepine binding in the cortex, without altering the stressor-induced increase in corticosterone. Thus, the mediation seemed to be neurally, rather than hormonally, mediated. Our animals were tested 5 minutes after the lidocaine lesion, which is too soon for elevations in corticosterone concentrations to have occurred. Overall, the data indicate that the dorsomedial hypothalamus acts via a neural pathway to reduce the sensitivity of the GABA-benzodiazepine receptor complex in other brain areas. Rapid allosteric changes of the receptor in hypothalamic projection areas such as frontal cortex, hippocampus, amygdala, and central periaqueductal grey may be a crucial mechanism underlying both the response on trial 2 in the plus-maze and the insensitivity to benzodiazepines. We have already shown that in the dorsal raphé nucleus (part of the ventral periaqueductal grey) a change in benzodiazepine receptors to an inverse agonist state is the mechanism underlying the trial 2 loss of response to benzodiazepines in this region (Gonzalez and File 1997).

Our previous experiments (File et al. 1998) showed that the basolateral nucleus of the amygdala plays a crucial role in the consolidation of information, acquired during trial 1 in the plus-maze, that in turn leads to insensitivity to benzodiazepines on trial 2. Thus, inactivation of the basolateral nucleus of the amygdala by lidocaine injections immediately after trial 1 allowed rats to show an anxiolytic response to chlordiazepoxide on trial 2, shown by both an increased percentage of entries onto open arms and an increased percentage of time spent on the open arms. Because the lidocaine injections produced only a temporary block of neurotransmission, the basolateral nucleus was fully functional at the time of testing on trial 2, which was 48 hr later. McGaugh et al. (1996) have proposed that the basolateral nucleus of the amygdala plays a prime role in regulating the consolidation of storage, in other brain areas, of information of conditioned motivational value. Our experiment did not address the roles of the basolateral nucleus of the amygdala in the acquisition or retrieval of this information, but on the basis of other studies (Parent and McGaugh 1994; Killcross et al. 1997) these roles are likely to be less crucial. The present experiment did not address the issue of consolidation since the dorsomedial hypothalamus was inactivated 48 hr after trial 1 and immediately before trial 2. Our present results show the importance of the dorsomedial hypothalamus in the retrieval of information that leads to animals losing sensitivity to benzodiazepines on trial 2. Importantly, the dorsomedial hypothalamus would seem to be mediating a change in the sensitivity of benzodiazepine receptors in other brain areas. If the dorsomedial hypothalamus is a site important for both the consolidation and retrieval of information acquired on trial 1, then it would be expected that inactivation immediately after trial 1 would also change the subsequent response to benzodiazepines on trial 2. However, this is most likely to affect only the escape components of the response and other brain regions are more likely to mediate the avoidance component.

Because we had too few placement errors for analysis, our experiment did not provide any information on the possible role of adjacent nuclei. We took the precaution of making the injections at 19° towards the midline, in order to avoid passing through the ventricles, mini-

mising the possibility of ventricular spread to other areas. The possible roles of other brain areas remain to be explored, but already the results of the present experiment, together with those of our previous experiment, highlight the importance of several different brain areas interacting to reduce the sensitivity to benzodiazepines on trial 2 in the elevated plus-maze.

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