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Shock-Induced Defensive Fighting in the Rat: Evidence for Cholinergic Mediation in the Lateral Hypothalamus

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BELL, R. AND K. BROWN. *Shock-induced defensive fighting in the rat: Evidence for cholinergic mediation in the lateral hypothalamus.* PHARMAC. BIOCHEM. BEHAV. 12(4) 487–491, 1980.—Bilateral microinjections of scopolamine into the lateral hypothalamus significantly reduced shock-induced defensive fighting, without altering jump threshold values. Further investigation of the lateral hypothalamus demonstrated that (1) fighting increased in response to bilateral microinjections of physostigmine and carbachol, (2) social attraction remained unaltered following scopolamine treatment, (3) neither motor co-ordination nor motor activity was significantly affected by any of the treatments.

Shock-induced defensive fighting Jump threshold Lateral hypothalamus Cholinergic mechanisms
Scopolamine Physostigmine Carbachol

EVIDENCE for central cholinergic control of aggression has come from studies employing peripheral administration of anticholinergics. For example scopolamine has been found to decrease isolation-induced aggression in mice [7], social aggression in rats [26,32], shock-induced defensive fighting in rats [24,25], and predatory attack in cats [13]. These anti-aggressive effects have been demonstrated over a wide phylogenetic range: scopolamine inhibited the attack behaviour of the ant, *Formica Rufa*, [16], of cichlids [1] and of squirrel monkeys [23]. Direct brain injections of anticholinesterases (physostigmine and amitone) and a cholinomimetic (carbachol) have demonstrated cholinergic mediation of mouse-killing in the lateral hypothalamus [3,29], medial thalamus [4], ventral midbrain tegmentum [5], and basolateral amygdala [11].

Bilateral microinjections of scopolamine in the basolateral amygdala, ventral and dorsal hippocampus significantly reduced levels of shock-induced defensive fighting in rats [28]. However, whilst jump threshold values remained unaltered in the amygdala group, both of the hippocampal groups exhibited decreased shock sensitivity. Because of the strong evidence implicating amygdaloid influence of hypothalamic output [9,19], it was of interest to determine the neurotransmitters within the hypothalamus involved in the mediation of aggression. The lateral nucleus of the hypothalamus was selected since it receives a strong cholinergic input from the basolateral amygdala via the ventral amygdalofugal pathway [10]. It was hypothesized that acetylcholine might play a similar role in the lateral hypothalamic control of shock-induced defensive fighting to that shown for the basolateral amygdala [28].

METHOD

Subjects

Adult male Sprague-Dawley rats obtained from a local supplier, (Olac Ltd.) and weighing approximately 300 g at the time of surgery, were used. They were individually housed with food and water available ad lib and maintained on a 12 hr light/dark cycle 6.00–18.00 hr. Testing was performed under red light during the dark phase of this cycle, from 18.00–22.00 hr.

Surgery

Cannulation. Experimental subjects were bilaterally implanted with guide cannulae under Equi-thesin (Jensen-Salsbury Lab., Inc.) anesthesia. Each cannula consisted of a 0.6 mm outer diameter (o.d.) stainless-stell guide fitted with a 0.3 mm o.d. obturator. The latter was removed and replaced by a 0.3 mm injection cannula prior to injection. The length of the injection cannula was such that when inserted into the guide, the tip of the cannula was flush with that of the guide.

Stereotaxic coordinates. Implanation coordinates (levelhead) for the lateral hypothalamus were 3.1 mm posterior to bregma, 1.7 mm lateral to the midline and 7.3 mm below the surface of the brain (-3.1 ± 1.7; 7.3 down). These values were calculated from the atlas of König and Klippel [15]. 10–14 days postoperative recovery was allowed before testing began.

Apparatus

Shock-induced defensive fighting. A Grason-Stadler rat station (E3125A...100), measuring 24×20×29 cm served as the test chamber. The chamber was opaque apart from the perspex door, which also served as an observation window. A Grason-Stadler shock generator (E1064) supplied scrambled shock of specified duration and intensity to the grid floor of the test chamber. The total number of shocks delivered in any series and the frequency of these shocks were controlled by relayed programming equipment (Grason-Stadler and Aim Biosciences).

Jump-flinch test. The same apparatus as that used for the Shock-Induced Defensive Fighting testing was employed in the screening of the animals for drug-induced changes in jump thresholds.

Rota-rod. This consisted of a kymograph recorder (C. F. Palmer), with drum removed, placed in a wooden cradle so that the centre spindle (rod) was horizontal. A thick wire mesh covering the rod provided a gripping surface for the animals. The apparatus was positioned so that the rod (length=31 cm; diameter=2.2 cm) was 70 cm above a thick cushion. The cushion, covered with tissue paper to absorb faeces and urine, was of ample thickness to safely arrest the fall of the animals. Rotation speed of this rota-rod was fixed at 4 rpm.

Activity box. This was constructed out of wood 58×58 cm sq and 30 cm high, with an aluminum floor tray. Ths walls of the box were painted matt white. Along two sides of the box were aligned ten photocells, spaced at 10 cm intervals and located 2 cm above the level of the floor tray. Each photocell was connected to a separate electrically activated counter, and a summation of these readings gave a measure of activity during the test.

Latané test. This test was originally devised by Latané [17] in order to study the effects of various forms of early experience on rat social behaviour. A circular arena 1.22 m in diameter with a 45 cm high wall was used. The floor and walls were painted matte white, with the former marked off into 49 equal areas by a series of concentric circles and radii. Each area was labelled with a code number for ease of recording.

Total contact time during each session was recorded on an electronic digital timer (Fourth Instruments) to the nearest 0.01 sec. A conventional electronic timer (Birkbeck) gave a visual signal every 10 sec throughout the test session.

Procedures

The four tests were carried out in a counter-balanced order, with an intertest interval of three days.

Shock-induced defensive fighting. Animals were randomly assigned to fighting pairs. Each pair was placed in the test chamber and allowed 5 min to adapt to each other and the new environment. After the habituation period, the shock trials commenced. The shock intensity was set at 2mA, the shock duration at 0.5 sec and the frequency at 10.5 sec (see ref. [31]). Sixty pre-injection shocks were administered as two series of 30 shocks with an inter-series interval of 60 sec. Then, the operated animal was removed from the chamber, injected bilaterally with the appropriate drug and immediately replaced in the test chamber. The injection cannulae were left in situ during the entire post-injection phase. Sixty post-injection shocks were then delivered to the pair, immediately after the return of the injected animal to the chamber.

Responses to shock were recorded as follows: (a) no reaction; (b) upright threat posture; (c) a fight response/attack. The fight response/attack was recorded when one animal made a direct movement towards the other, either by lungeing with its whole body or by making a striking motion with its forepaws. A simple upright threat posture was not recorded as a fight response. Attack frequency was calculated only for the operated animal in each pair.

Jump-flinch test. Operated animals were placed individually in the test chamber and received 0.5 sec scrambled shock delivered to the grid floor at 15 sec intervals. Within a test series, shock intensities in the range 0.2–1.6 mA were delivered in 10 steps. Three ascending series alternated with three descending series and the jump threshold—the intensity at which the animals' hind feet left the grid floor—was determined for each series. The animal was then removed from the test chamber, injected bilaterally and replaced. Retesting commenced immediately and a post-injection jump threshold was determined, again using three ascending and three descending series of shocks. Data reported are the arithmetic means of the six trials.

Rota-rod. Animals were trained to remain on the rotating rod for 60 sec to a criterion of 4/5 trials (intertrial interval, 10 sec). Animals were then tested again for a criterion of 60 sec on 4/5 trials, under the various treatment conditions.

Activity test. Operated animals were placed individually in the activity box and allowed a period of 5 min to habituate to the new environment. The photocells were then switched on and the activity over the next 5 min was noted. Interruptions of each photobeam were recorded on separate counters and the readings were summated to give an overall activity score for the test period. This procedure was repeated under the various experimental treatments.

Latané test. Pairs of rats (one operated, one unoperated) were placed in the circular arena for a 5-min period and the position of each animal was recorded every 10 sec. Total contact time between the pair was also noted over the test session. A computer program was used to calculate the distance travelled by each animal and the mean distance maintained between the pair over the session. The effects of various treatments were again studied on this test.

Drugs

Drug doses were calculated on the basis of those used in previous studies (eg. [18,28]). Scopolamine hydrobromide (Sigma Chemical Co.) was made up in a solution of concentration 10 mg/ml. Physostigmine salicylate (Sigma Chemical Co.) and carbachol chloride (Sigma Chemical Co.) were made up in a solution of concentration 5 mg/ml. Normal physiological saline served as the vehicle for each drug and as the injection control.

Histology

Cannulated animals received an overdose of Nembutal and were perfused with normal physiological saline followed by 10% formal saline. Brains were removed, embedded in wax and serial sections cut at 10 μ. Sections were stained for myelin (Luxol Fast Blue), counter-stained for cell bodies (Pyronin Y), mounted and examined for cannula tracts with reference to the atlas of König and Klippel [15]. In addition, slides were carefully examined to determine the extent of tissue damage.

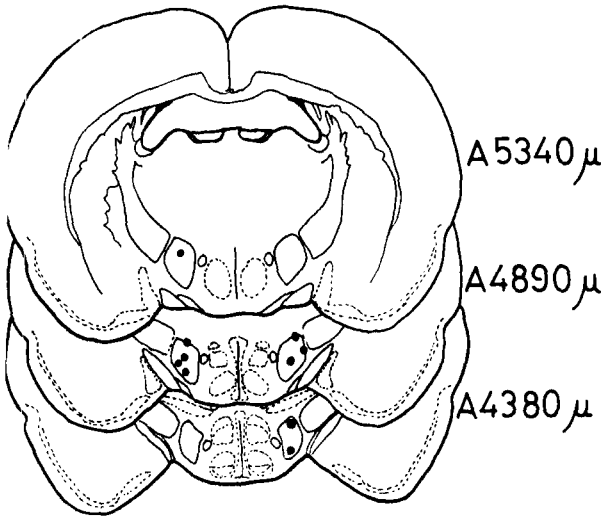

FIG. 1. Schematic summary of cannulae tip placements in the lateral hypothalamus for animals in Experiment 1.

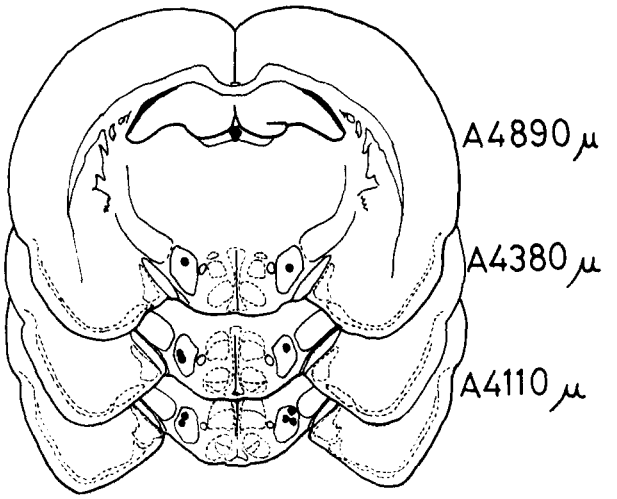

FIG. 2. Schematic summary of cannulae tip placements in the lateral hypothalamus for animals in Experiment 2.

EXPERIMENT 1

Electrolytic lesions [22] and procaine anaesthesia [6] of the lateral hypothalamus produced a blockade of shock-induced defensive fighting. These effects were not accompanied by changes in jump thresholds. The anticholinergic agent scopolamine hydrobromide (1 μl bilateral injections; 10 μg/μl) was used to examine whether cholinergic mechanisms might play a role in the behavioural effects seen following irreversible and reversible lateral hypothalamic lesions. In an analysis of the regional distribution of the enzyme choline acetyltransferase in the rat hypothalamus, high levels of the enzyme (indicative of acetylcholine levels) were found in the lateral hypothalamus [30].

Ten rats were randomly assigned to two groups. Group 1 (n=5) were bilaterally implanted with guide cannulae aimed at the lateral hypothalamus; Group 2 (n=5) served an unoperated fight opponents.

Histology

Figure 1 gives a diagrammatic summary of the histological verification of cannulae tip placements.

RESULTS

Two-tailed correlated t-tests were used on the aggression and jump threshold results to compare postinjection responses with preinjection baselines. Table 1 shows that scopolamine, when injected into the lateral hypothalamus, produced a significant reduction in shock-induced defensive fighting, $t(4)=6.04$, $p<0.005$, while saline was without effect, $t(4)=0.41$, NS. Jump thresholds were unaltered following injections of either scopolamine, $t(4)=1.10$, NS, into the lateral hypothalamus. Neither scopolamine nor saline had any effects on locomotor co-ordination (criterion: 60; saline: 60; drug: 60) or open field ambulation, $F(3,12)=0.61$, NS.

The results of this experiment suggest that, in the lateral hypothalamus of the rat, central cholinergic (muscarinic) mechanisms may mediate shock-induced defensive fighting.

TABLE 1

MEAN ATTACK SCORES PER 60 SHOCKS (± SD) FOR INJECTED ANIMALS IN EXPERIMENT 1.

Treatment	Preinjection baseline	Postinjection response
Scopolamine	28.2 ± 6.1	11.4 ± 7.5*
Saline	32.0 ± 6.0	32.4 ± 7.1

*$p<0.005$.

EXPERIMENT 2

Bilateral injections of physostigmine into the lateral hypothalamus have been reported to significantly increase muricide [3,29] and shock-induced defensive fighting [6]. Lateral hypothalamic injections of crystalline carbachol also elicited killing in rats that would not ordinarily kill mice [29]. From the results of Experiment 1, it was predicted that potentiation of synaptic ACh, by physostigmine (1 μl, 5μg/μl) or the application of a cholinomimetic, such as carbachol (1 μl, 5μg/μl), should produce the opposite effect. Ten rats were randomly assigned to 2 groups; group 1 (n=5) were bilaterally implanted with cannulae aimed at the lateral hypothalamus, while group 2 (n=5) served as unoperated fight opponents. One experimental animal died reducing the number of pairs to 4.

Histology

Figure 2 illustrates cannulae tip placements in the lateral hypothalamus for the second experiment.

RESULTS

One-tailed correlated t-tests were used to analyse the data. Table 2 shows that carbachol produced a significant increase in shock-induced defensive fighting, $t(3)=5.83$, $p<0.01$, as did physostigmine, $t(3)=15.54$, $p<0.005$. Saline produced no significant effect on this form of aggression, $t(3)=1.67$, NS.

TABLE 2

MEAN ATTACK SCORES PER 60 SHOCKS (± SD) FOR INJECTED ANIMALS IN EXPERIMENT 2

Treatment	Preinjection baseline	Postinjection response
Carbachol	21.3 ± 6.3	32.5 ± 7.1*
Physostigmine	21.8 ± 3.0	32.8 ± 3.1†
Saline	24.0 ± 7.0	24.8 ± 6.9

*$p<0.01$.
†$p<0.005$.

Jump threshold values were unaltered by injections of either carbachol, $t(3)=0.17$, NS, physostigmine, $t(3)=0.09$, NS, or saline, $t(3)=1.13$, NS.

This was also true for locomotor co-ordination (criterion: 60; saline: 60; carbachol: 60; physostigmine: 60) and open field ambulation, $F(3,9)=0.42$, NS.

This experiment provided further evidence for a central cholinergic mechanism involving the lateral hypothalamus which appears to mediate shock-induced defensive fighting.

EXPERIMENT 3

Rodent social behaviour, as measured by open-field contact time, may be disrupted by electrolytic lesions to the hippocampus [14] and amygdala [12,14]. Such findings have been explained in terms of reduced responsiveness to social stimuli. However, scopolamine injections into the basolateral amygdala did not alter social attraction in rats [28].

Experiment 3 was designed to test the possibility that bilateral scopolamine injections (1 μl, 10 μg/μl) into the lateral hypothalamus might decrease general social responsiveness in rats. Using a test of social attraction [17], it was possible to measure various components of social behaviour between paired rats placed in a large open arena.

Ten rats were randomly assigned to two groups. Group 1 (n=5) were bilaterally implanted with cannulae aimed at the lateral hypothalamus. Group 2 (n=5) served as unoperated stimulus animals. A counterbalanced order was employed to prevent any habituation effects.

Histology

Figure 3 represents cannulae tip locations in the lateral hypothalamus.

RESULTS

ANOVA revealed that there were no significant differences between the different treatments on mobility, $F(2,8)=0.63$, NS, contact time, $F(2,8)=0.49$, NS, or mean distance, $F(2,8)=1.51$, NS.

These results indicate that social attraction between paired rats remained unaffected by bilateral scopolamine injections into the lateral hypothalamus. The data from Experiment 1, on the effects of scopolamine on locomotor activity, provide useful control results concerning the effects on cholinergic blockade in the lateral hypothalamus.

GENERAL DISCUSSION

Bilateral microinjections of the cholinergic antagonist scopolamine HBr produced a significant reduction in shock-induced defensive fighting, with no alterations in jump thresholds, locomotor activity or co-ordination. This result was interpreted as preliminary evidence suggesting the role of ACh as a substance mediating this type of aggression in the lateral hypothalamus.

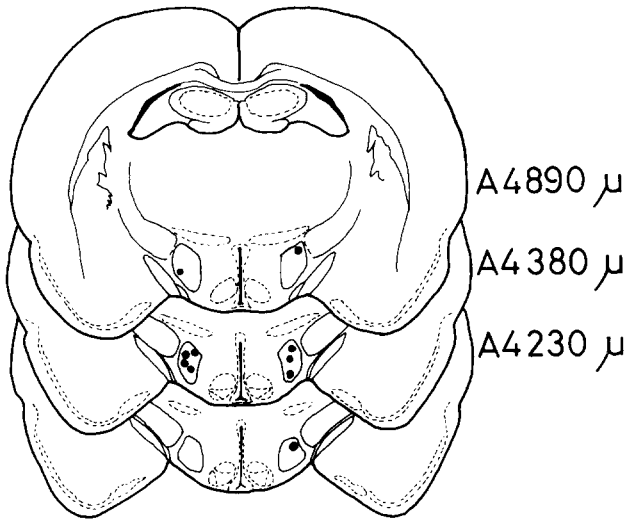

FIG. 3. Schematic summary of cannulae tip placements in the lateral hypothalamus for animals in Experiment 3.

Further evidence for cholinergic control of shock-induced defensive fighting was obtained from bilateral microinjections of a cholinomimetic, charbachol, and an anticholinesterase physostigmine salicylate.

Both compounds produced a significant increase in aggression without any concomitant effect on jump thresholds or activity.

Disruption of social behaviour was tested in the Latané test of social attraction. Scopolamine had no effect on the various measures of social attraction, compared to controls.

Comparing the findings of Bandler [2, 3, 4, 5] on the cholinergic mediation of muricide, with those of Rodgers [27] and the present study on shock-induced defensive fighting, it is suggested that the lateral hypothalamus and basolateral amygdala may both be involved in the central cholinergic mediation of these two types of aggressive behaviour. Cholinergic facilitation of shock-induced defensive fighting may be mediated from the basolateral amygdala, via the ventral amygdala-fugal pathway to the lateral hypothalamus [27]. From the hypothalamus, effector mechanisms, mediated by ACh, may operate through lower brain structures such as the midbrain central grey and the ventral midbrain tegmentum. In fact, the midbrain central grey may act as a modal command system on these mechanisms [8].

Another control necessary to demonstrate drug specificity in the lateral hypothalamus concerns the problem of drug diffusion to other sites. Injections of radioactively labelled neurotransmitters has been found to remain in the hypothalamic block for 30 min postinjection [21]. Dye diffusion studies [20] have shown that diffusion tends to be along the cannulae tracks (a result confirmed in dye studies in this investigation). Preliminary examination of a control site 1 mm dorsal to the lateral hypothalamus, the ventral thalamus, suggests that drug diffusion to this structure does not influence shock-induced defensive fighting. (Bell and Brown, unpublished results).

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