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Nucleus accumbens lesions impair context, but not cue, conditioning in rats

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PREVIOUS work has provided evidence of a role for the hippocampal formation in contextual as opposed to cue conditioning. Similar deficits have been observed after transection of the fimbria/fornix, part of which consists of the hippocampal-nucleus accumbens (N.Acc) connection arising from both the dorsal and ventral subiculum. By means of electrolytic lesions of the N.Acc, we showed that the subiculo-accumbens projection appears to participate in aversive conditioning to context, but not to a cue (tone). Freezing, measured as an index of learning, in the experimental context was greatly reduced in animals with lesions of the N.Acc, as compared with sham-operated controls. No difference was found in freezing to a distinct tone. These data lend further support to the notion that the N.Acc is an important interface between limbic structures and motor output.

Key words: Conditioning; Context; Cue; Hippocampus; Lesions; Nucleus accumbens (N.Acc)

Introduction

The hippocampal formation has been implicated in various behavioural learning paradigms, including spatial learning and contextual conditioning. Examination of the effects of electrolytic lesions of the dorsal hippocampus in contextual fear conditioning paradigms has demonstrated both retrograde[1] and anterograde performance deficits.[2,3] These lesions did not, however, affect conditioning to explicit cues such as a tone.[2] The mechanisms responsible for these processes, however, are unclear. In order to achieve a more detailed functional understanding of the hippocampus, one has to consider the anatomical connections of the hippocampus and their contribution to the specific learning tasks. Anatomically, one major input pathway to the hippocampal formation is the perforant path connection from and to the entorhinal cortex. Evidence, however, suggests that this pathway is of minor importance, since lesions of the entorhinal cortex do not result in learning impairments in contextual conditioning.[4,5] In contrast, transection of the fimbria/fornix caused impaired contextual conditioning,[4] suggesting a potential role of subcortical inputs and outputs of the hippocampus in context conditioning. The major input of fimbria/fornix fibres arises from the medial septum, lesions of which have been reported to potentiate contextual conditioning,[6,7] supporting the importance of this input.

Information about hippocampal output targets and their potential role in contextual conditioning, however, remains sparse. Hippocampal efferents issue via the fimbria/fornix to several regions in the basal forebrain. Fibres arising in dorsal and ventral parts of the subiculum have been reported to terminate in the dorsal (caudate-putamen) and ventral striatum (nucleus accumbens, N.Acc).[8] The latter connection is glutamatergic[9] and high frequency stimulation of the fimbria/fornix or subiculum can lead to long-term potentiation of N.Acc responses.[10–12] Recordings of cells in the N.Acc from freely moving rats show place-specific firing[13] and lesions of the N.Acc produce deficits in both delayed matching-to-position operant conditioning[14] and spatial win-shift radial arm maze training.[15] It is of interest, therefore, to determine whether hippocampus-mediated freezing to context is also processed via the subiculo-N.Acc pathway. The present study was aimed at investigating this possibility.

Material and Methods

The subjects were 24 naive male hooded Lister rats (350–450 g, Harlan UK, Ltd). Twelve animals received bilateral electrolytic lesions of the nucleus accumbens (the specific procedures employed being those described below) with the intention of carrying out a HPLC analysis of lesion-induced effects on the level of central dopamine concentration. Subsequently that experiment was abandoned and the rats became available for this investigation. The original experiment (unlike the present experiment) had not required an operated control group; at this point,

therefore a batch of 12 animals, matched for age with the experimental group, received sham lesions (see below). The rats were housed in pairs with continuous access to food and water throughout the experiment in holding rooms temperature-controlled to 21 ± 2°C.

The rats were anaesthetized with avertin (made up as 1.25 ml avertin concentrate added to 5 ml absolute alcohol and 62.5 ml physiological saline) administered at 10 ml/kg, i.p. (Avertin concentrate consists of 100 g 2,2,2,tribromoethanol dissolved in 62 ml tertiary amyl alcohol.) The top of each animal's head was shaved and washed with an antiseptic solution. The animal was then placed in a stereotaxic frame (Trent Wells, California, USA). The skin and tissue was reflected from the skull and two small holes were made using a dental drill (Hudson Ltd.) above the intended lesion site. The lesion coordinates, taken from a level skull, were 2 mm anterior to Bregma, 1 mm lateral to the midline and 66 mm ventral to the brain surface. The electrode was positioned and then lowered on the arm of the stereotaxic frame in accordance with the lesion coordinates. Lesions were produced by passing a 1 mA current from a constant-current lesion maker for 15 s through a wire electrode, insulated to within 0.5 mm of its tip. For sham-operated subjects the procedure was the same except no current was passed.

After behavioural testing had been completed, animals were deeply anaesthetized with pentobarbitone sodium and perfused intracardially with physiological saline followed by 10% formol–saline. The brains were removed and stored in a sucrose formalin solution for 2 weeks before being frozen and then sectioned in the vertical plane (30 μm). Sections were retained at 150 μm intervals throughout the lesioned area. They were then mounted and stained with cresyl violet. The sections from each animal were inspected under a light microscope and representations of lesion-induced damage were transferred onto plates from a stereotaxic atlas[16] located at positions 0.48, 0.70, 1.20, 1.70 and 2.20 mm anterior to bregma. At each location the maximum and minimum extent of the lesion was depicted. Verification of lesion placement was made on the basis of these reconstructions.

Contextual versus cue conditioning essentially followed the method described previously.[2,17] In brief, on day 0, animals were habituated to the conditioning chamber (25 × 21 × 19 cm) for 20 min. On each of the following 2 days rats were subjected to one acquisition session consisting of two trials (intertrial interval 60 s), which were video-recorded. After 2 min of adaptation, a period of 20 s prior to the conditioned stimulus (CS) was recorded as context (pre-CS) followed by a 20 s CS (tone, 10 kHz, 80 dB) the termination of which was followed immediately by a 500 ms scrambled footshock (0.25 mA). After the second trial, rats remained in the conditioning chamber for a further 30 s and were then returned to their home cages. On day 3, only one test trial was given, which was not followed by a shock.

Freezing (complete absence of movements except respiratory activity) was continuously scored for both pre-CS and CS periods by an experimenter blind to the condition of the animals. Particular weight was given to freezing in trial one of each session, which reflects the learning that had occurred as a result of conditioning on the previous day(s).

In an attempt to assess possible non-specific effects of lesion-induced damage (e.g., changes in locomotor activity) animals were subjected to two further behavioural measures. First, locomotor activity, measured as lateral and vertical movements when the rat's snout crossed lines bisecting upper and lower, and right and left, halves of the front opening of the conditioning chamber were scored for the first 5 min of the session on day 0. Second, after completion of the conditioning task, the animals' locomotor activity was recorded in eight identical activity boxes (60 × 60 × 30 cm) equipped with two sets of infra-red beams, interruption of which was remotely recorded and stored on disc. Beam breaks of the lower set recorded lateral, breaks of the upper set recorded vertical movements. Activity was monitored over the course of a 60 min session and the scores pooled into 5 min bins.

Results

Figure 1 presents coronal sections through the rat brain on which are superimposed reconstructions of the electrolytic lesion-induced damage. It shows that the animals suffered considerable bilateral damage to the N.Acc. This damage was mainly restricted to the shell region of the structure, with minimal damage to the core area, the ventral pallidum, lateral septal nucleus and the medial forebrain bundle. One of the experimental animals (not shown) had insufficient lesion damage and was excluded from the behavioural analyses. The operated control animals showed a small degree of cortical damage caused by electrode placement but there was no apparent damage to the N.Acc itself. Therefore all animals in the sham group were included in the behavioural analyses.

Conditioning to context was retarded in N.Acc-lesioned animals compared with sham-lesioned controls (Fig. 2, upper panel). Both groups showed very little freezing during the pre-CS of day one of acquisition training. Although freezing increased in the N.Acc group on days 2 and 3, performance was well below that seen in the sham group. An analysis of variance (ANOVA) with lesion and day as factors

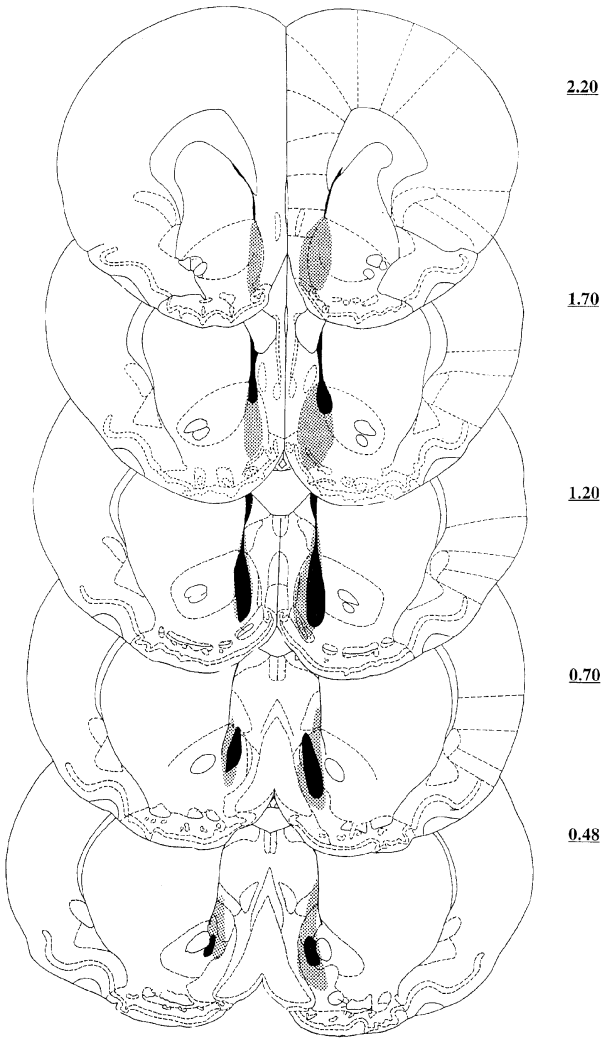

FIG. 1. Reconstructions of the lesion-induced damage superimposed onto plates from the atlas of Paxinos and Watson.[16] The filled area shows the minimum extent of the lesion and the stippled area shows the maximum extent. Figures denote positions anterior to bregma (mm).

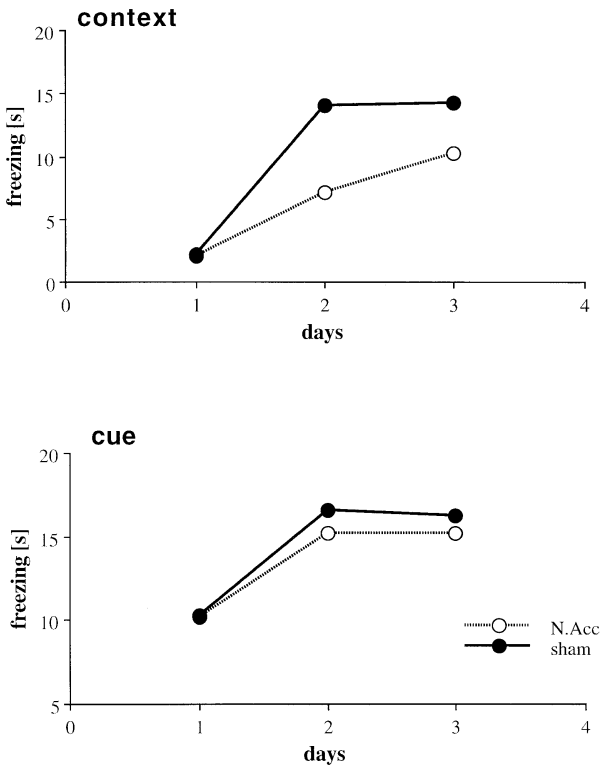

FIG. 2. Mean number of seconds spent freezing on trial one in the presence of contextual stimuli (upper panel) and the explicit conditioning stimuli (lower panel) for 3 experimental days.

revealed a main effect of lesion ($F(1,21) = 6.6$, $p < 0.02$) and a main effect of day ($F(2,42) = 28.0$, $p < 0.0001$), but the interaction failed to reach conventional levels of significance ($F(2,42) = 2.8$, $p > 0.07$). These results therefore confirm a performance deficit in contextual conditioning following N.Acc lesions. *Post hoc* simple main effect analysis, however, revealed that, although the groups differ in the amount of freezing, both groups did improve their performance over days ($p < 0.001$ for both groups), suggesting that contextual fear conditioning is attenuated but not abolished in N.Acc-lesioned animals.

Freezing to the explicit cue did not differ between groups (Fig. 2, lower panel). An ANOVA with lesion and days as factors revealed a significant main effect of day ($F(2,42) = 15$, $p < 0.0001$), but not of lesion ($F < 1$), similarly there was no lesion-by-day interaction ($F < 1$). Interestingly, freezing during the CS period of the first acquisition trial of day one was relatively high, although no footshock had been delivered at that stage. This presumably reflects unconditioned suppression of responding to a novel stimulus (in this case, to the first appearance of a high pitch tone).

Since freezing is an activity-dependent process, and pharmacological blockade of glutamate receptors in the N.Acc has previously been shown to reduce locomotor activity,[18,19] we investigated the possibility that our lesion might have a direct effect on locomotor activity. Measurement of lateral and vertical movements during the first 5 min of training on day 0 revealed no differences between the groups on either measure ($F < 1$). The mean lateral crossings in the N.Acc and sham groups were 10.7 ± 0.5 (mean ± s.e.m.) and 10.1 ± 0.6, and vertical crossings were 8.3 ± 1.1 and 9.3 ± 0.6, respectively. These data indicate no activity differences between the two groups in the conditioning chamber. Support for this conclusion comes from the scores obtained from the final test in the activity boxes. Figure 3 summarizes the results for both lateral (upper panel) and vertical activity (lower panel). Both groups showed habituation as indicated by a continuous decrease in activity

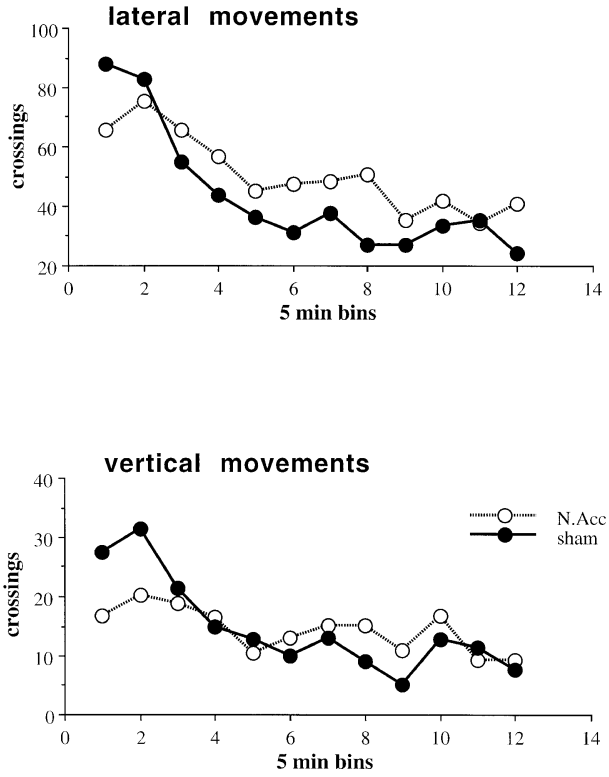

FIG. 3. General locomotor activity of sham and N.Acc-lesioned animals in the activity box during 60 min. Infra-red beam crossings of the lower array were scored as lateral movements (upper panel) and crossings of the upper array were scored as vertical movements (lower panel).

over time. ANOVA with lesion, direction of movement, and bins as factors revealed a main effect of direction of movement ($F(1,42) = 149.1$, $p < 0.0001$) reflecting the difference between lateral and vertical activity, and a main effect of bin ($F(11,462) = 23.3$, $p < 0.0001$). There was no main effect of lesion ($F(1,42) = 1.6$, $p < 0.2$), no lesion-by-direction of movement interaction ($F(1,42) = 2.1$, $p > 0.1$), and no lesion-by-direction of movement-by-bin interaction ($F < 1$). There was, however, a lesion-by-bin interaction ($F(11,462) = 3.7$, $p < 0.0001$) and a subsequent examination of the simple effects revealed a significant reduction in activity in the N.Acc group during the first 5 min bin ($p < 0.001$). These data suggest that N.Acc-lesioned animals show somewhat less activity than control animals when first exposed to a novel context.

Discussion

Contextual conditioning has been shown to depend on the functional integrity of the hippocampal formation,[20] whereas lesions to both parts of or the entire hippocampal formation do not affect classical conditioning to an explicit stimulus such as a tone. The paradigm used here essentially replicates the procedure introduced previously by Phillips and LeDoux,[2] in studies of the hippocampus, and although we applied a weaker shock intensity (0.25 mA instead of 0.5 mA) we have generated similar results with hippocampal animals (unpublished data), namely a complete abolition of conditioning to context, but not to cue. This demonstrates that our paradigm, like that of Phillips and LeDoux,[2] is capable of distinguishing between hippocampus-dependent and -independent conditioning tasks.

Our main interest, however, was in the major efferent pathways conveying the information required for correct performance in this combined context and cue conditioning task. Since lesions of the entorhinal cortex do not affect performance in either conditioning paradigm,[4,5] fibres back projecting from hippocampal CA1/subiculum to the entorhinal cortex are probably not involved in contextual conditioning. Thus, a subcortical route seems to be implicated, and since the deficit induced by lesions of the fimbria/fornix resembles the hippocampal deficit in this paradigm, efferents conveying information to particular parts of the basal forebrain may be involved. One possible target, of course, is the lateral septum. Electrophysiological recordings from the lateral septum in freely moving mice submitted to shock reinforced contextual conditioning showed a context-specific reduction in the N3 component of the synaptic response upon re-exposure to the conditioned environment.[21] These data support a possible involvement of the lateral septum in contextual conditioning.

Alternatively, efferent fibres of the subiculum, which constitute part of the fimbria/fornix system, may carry information to the dorsal and/or ventral striatum (caudate-putamen complex and/or nucleus accumbens, respectively).[8] We were particularly interested in the latter pathway, because the N.Acc occupies a strategic position as an interface between limbic cortex and midbrain areas involved in motor output. Moreover, the subiculo-N.Acc pathway has the capacity to express long-term potentiation,[10–12] a form of cellular neuronal plasticity widely believed to be mirrored in some forms of learning and memory. Our data show that N.Acc lesions disrupt the acquisition of freezing to conditioned cues while leaving learning about an explicit cue unaffected. Although the attenuation in conditioning to context was significant, it was far from complete, as has been previously described for hippocampal animals.[1,2] This suggests that hippocampal efferents to other brain areas also take part in freezing to context. The targets for these efferents remain elusive, but one possible candidate might be the dorsal striatum (caudate-putamen) which receives extensive input

from the dorsal and ventral subiculum,[8] and is involved in the modulation of various forms of behaviour.

Previous work has suggested that the N.Acc may play a role in modulating locomotor activity.[16,17] Hence, we investigated whether N.Acc lesions cause alterations in locomotor activity to novelty, which could account for a deficit in freezing to context. Although we found a slight reduction in activity as measured during habituation in the activity cage, this could not be responsible for the impaired performance in contextual conditioning for two reasons: first, because reduced activity would generally favour an increase in freezing; second, because a general change in activity should affect freezing to both context and cue.

Conclusion

Overall, our data provide the first evidence for a potential role of the N.Acc in contextual as opposed to cue conditioning. Although hippocampal lesions have been shown to seriously impair contextual conditioning, lesions of the N.Acc produce a less severe attenuation of the effect. Since the subiculo-N.Acc projection constitutes a part of the fimbria/fornix fibre system, lesions of which also prevent conditioning to context, it seems reasonable to argue that this efferent pathway of the hippocampus participates in mediating contextual conditioning. However, it is not the only connection between limbic cortex and midbrain motor structures, and further work is required to elucidate which alternative routes are required for performance in contextual conditioning, and whether these structures act in parallel or in concert.

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General Summary
There is a pivotal interest in understanding the function of particular brain regions in mechanisms of learning and memory, especially because several forms of human dementias are paralleled by cognitive deficits. The hippocampus, which is part of the limbic cortex, is known to be essential for spatial cognitive mapping and learning about context (environment). We show here that one possible output pathway, by which information may be carried in order to create motor responses, is the hippocampus–nucleus accumbens projection. Lesions of the nucleus accumbens in rats produce an impairment in learning about the context, but not about an explicit tone. However, lesioned animals still show some learning suggesting that the nucleus accumbens may constitute only one target of hippocampal efferents. Work is currently in progress to identify additional hippocampal output structures and their role in learning about context.