VENTRAL TEGMENTAL AREA LESIONS DIFFERENTIALLY AFFECT RESPONSES CONTROLLED BY CS-US CONTIGUITY AND RESPONSE-REINFORCER CONTINGENCY IN THE RAT

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Abstract. Rats with electrolytic lesions of the ventral tegmental area (VTA, \(n=32\)), or sham lesions (\(n=32\)), were tested in four behavioral paradigms in a shuttlebox. The paradigms were designed to assess the motivational influence of CS-US contiguity and response-reinforcer contingency. The VTA lesion increased the number of shuttle responses in the paradigms involving contiguity between a warning signal and a shock, without affecting responses controlled by the contingency between shuttling and shock avoidance. Escape latency was reduced by the lesion, but the general locomotor activity of the rats was not increased. The data suggest that the VTA normally acts to inhibit behavior motivated by classically conditioned fear. The results are discussed in terms of the hypothesis that the dopaminergic projection from the VTA to the nucleus accumbens plays a role in the inhibition of emotionally motivated motor responses.

INTRODUCTION

Electrolytic lesions of either the nucleus accumbens or the ventral tegmental area, source of the dopaminergic projection to the accumbens, are known to enhance active avoidance performance in rats (3, 5, 9). This effect could result from a general disinhibition of motor activity,
or could arise because of a more specific motivational, emotional, or cognitive effect of the lesion. Mogenson, Jones, and Yim (6) have proposed that the accumbens acts as an interface between emotional and motor systems. This hypothesis suggests that the enhancement of avoidance behavior might result from a specific emotional effect of the lesion.

Izquierdo and his colleagues have suggested that three factors contribute to the responses made by a rat in a two-way signalled avoidance paradigm. The occurrence of an aversive stimulus (shock) gives rise to generalized drive or arousal. The contiguous presentation of the signal and shock leads to a classically conditioned fear of the warning signal. Finally, the contingency between the response and shock absence reinforces responses that occur during the signal. Izquierdo has suggested that these three factors are independent, and that they alone can account for all of the responses made by the rat in the two-way avoidance situation. (See (2), for a review).

The extent to which these factors contribute to the response can be determined by comparing the number of responses made by rats in situations in which the signal-shock contiguity and the response-shock contingency are manipulated. In the absence of both signal-shock contiguity and response-shock contingency, the generalized drive produced by the shock motivates shuttling. The addition of either contiguity or contingency or both increases the underlying motivation, and thus the number of shuttle responses. By manipulating these factors, Schutz and Izquierdo (9) determined that the enhancement of avoidance responding that follows electrolytic lesions of the accumbens is due to an increase in generalized drive. That is, even in the absence of signal-shock contiguity and response-shock contingency, rats responded more to the warning signal.

However, manipulations that enhance baseline locomotor activity without specifically affecting generalized drive produced by the shock would have the same effect; that is, the rats would shuttle more. The GABAergic projection from the accumbens to the ventral pallidium is known to inhibit motor activity (7); the removal of this projection via the electrolytic lesion could therefore disinhibit locomotion without enhancing drive. Izquierdo’s procedure cannot differentiate between these possibilities.

Disinhibition of motor activity might also explain our earlier finding that electrolytic lesions of the accumbens facilitate classically conditioned leg flexion in the cat (11). The infusion of dopamine into the accumbens attenuates this conditioned motor response (12), despite the fact that locomotor activity in an open field setting is enhanced by dopamine. These data suggest that mesolimbic dopamine selectively inhibits classi-
cally conditioned motor responses. Therefore, VTA lesions, which would deplete the accumbens of dopamine, might enhance avoidance performance by disinhibiting those responses controlled by the contiguity between signal and shock. This hypothesis was tested by examining the effects of VTA lesions on shuttle performance while signal-shock contiguity and response-shock contingency were manipulated according to Izquierdo’s procedure. Additional measures were recorded to assess the contributions of general motor activity.

METHODS

Subjects. The experiment was conducted using 64 female Sprague-Dawley rats, obtained from Holtzman Company (Madison, WI). The rats weighed approximately 300 g at the time of surgery. They were housed in individual cages in a light-cycled room (12 h light, 12 h dark), with ad lib access to food and water throughout.

Surgery. Electrolytic lesions were produced bilaterally in the ventral tegmental area in 32 randomly-selected rats; the other 32 rats received sham surgeries. Each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal, 42 mg/kg) in conjunction with atropine sulfate (0.01 mg in 0.02 ml), and received an intramuscular injection of an antibiotic (Combiotic (Pfizer), 0.5 ml). In the VTA Lesion group, a stainless steel electrode, insulated except at the tip with Epoxylite, was inserted into the center of the ventral tegmental area (4.8 mm posterior to Bregma, ± 1.0 mm lateral to the midline, and 8.4 mm ventral to skull surface, according to the atlas of Paxinos and Watson, (8)). A lesion was produced by the passage of 1.0 mA anodal dc current for 10 s from the electrode to an anal cathode. In the Sham Lesion group the electrode was dropped to a point 2 mm dorsal to the VTA; no current was passed in this group. The rats were allowed at least one week to recover following surgery before testing was begun. One rat in the lesion group died between the first and second behavioral tests. Data from that rat are not included in the analyses.

Apparatus. Avoidance training was conducted in a Plexiglas shuttle-box (44.3 × 22.5 × 21.5 cm), housed in a ventilated, sound-attenuating chamber constructed from an Igloo picnic chest. A 24 V white light mounted in the top of this chamber illuminated the shuttlebox, and the ventilating fan provided masking noise. The shuttlebox walls were transparent, and the floor consisted of 5 mm stainless steel rods, spaced 15 mm center to center. This shuttlebox tilted around the central rod, and the tilting of the box activated a magnetic switch. Activation of this switch was recorded as a shuttle response.
A 24 V Sonalert speaker mounted in the center of the top of the sound-attenuating chamber was used to produce the auditory stimulus (tone).

A custom-built neon-scrambled shock generator could deliver shock stimuli to the floor of the shuttle box.

A DEC PDP-8 computer running SuperSked software (State Systems, Inc., Kalamazoo, Michigan, USA) controlled the delivery of stimuli and recorded responses made by the rats.

Procedure. Separate groups of VTA- and sham-lesioned rats were exposed to four different behavioral paradigms (based on those of Izquierdo, (1)), in which the Contiguity between the tone stimulus and the shock, and the Contingency between shuttling during that stimulus and shock delivery, were manipulated. In all four paradigms, the tone was presented to the rat in the shuttlebox 50 times, each with a duration of 5 s and with a randomly varied intertrial interval of 10-40 s. In each session of 50 trials, the number of trials on which the rat made a shuttle response during the tone was recorded.

In the Contiguous conditions, a 1.5 mA scrambled foot shock was scheduled to be delivered immediately at the offset of the tone. In the Noncontiguous conditions, the shock was scheduled to occur at a random 5-30 s interval following tone offset. In all conditions, shock remained on until the rat escaped by shuttling or for 5 s in the absence of an escape response.

One half of the rats in each of the Contiguous and Noncontiguous conditions experienced a Contingency between shuttling during the tone presentation and shock delivery: the shuttle response avoided the next scheduled shock, whether it was Contiguous or Noncontiguous. For the other rats, in the Noncontingent conditions, shock delivery occurred on schedule regardless of the rat's behavior during the tone presentation.

This crossing of Contiguity and Contingency factors resulted in four different behavioral conditions:

Noncontiguous, Noncontingent: Tones and shocks were presented in an unpaired manner; shuttle responses had no effect on shock delivery.

Contiguous, Noncontingent: Tones and shocks were explicitly paired; shuttle responses had no effect on shock delivery.

Noncontiguous, Contingent: Tones and shocks were presented in an unpaired fashion; shuttle responses during tone presentation avoided the next shock.

Contiguous, Contingent: Tones and shocks were explicitly paired; shuttle responses avoided the next shock. This is the traditional signalled two-way avoidance paradigm.
We chose to hold the number of trials received by rats in all conditions constant at 50. Consequently, the number of shocks experienced by rats in each condition varied. While possibly affecting comparisons across conditions, these differences do not affect the comparisons of interest, those between VTA- and sham-lesioned rats in the same condition. Rats in the Contiguous, Noncontingent condition always received 50 shocks. The number received by rats in the Contingent conditions depended on the number of avoidance responses made. We adopted Izquierdo's standard procedure (2, 9) of delivering shocks following 25 of the tone presentations in the Noncontiguous, Noncontingent condition (5 randomly selected tones out of each block of 10). Vendite, Elisabetsky, de Souza, and Izquierdo (10) have demonstrated that in this condition, the number of responses made does not vary as the number of shocks ranges from 10 to 50 per session.

In all four conditions, the number of shuttle responses made during the rat's first 3 min in the shuttlebox in the first session served as an index of general locomotor activity. The presentation of tones began after this 3 min period was over.

The latency to escape any unavaoided shocks was also measured in each of the paradigms, providing an index of the reactivity of the rat to the shock.

Each rat received two sessions of exposure to the paradigm, separated by one week. Following the last session, the rats were sacrificed with an overdose of sodium pentobarbitol, then perfused transcardially with isotonic saline and 37% formalin. The brains were removed, embedded in gelatin, frozen, and sectioned at 80 μm to allow an examination of the lesion site.

RESULTS

Histology. Examination of the brain sections revealed that the lesion destroyed much of the VTA, and sometimes encroached on more medial regions. The more lateral substantia nigra was not damaged. Fig. 1 illustrates the maximal extent of the lesion in the subjects with the smallest and largest lesions. In some subjects, a poorly insulated electrode caused extensive damage dorsal to the VTA and minimal VTA damage. The data from these subjects were eliminated from consideration, leaving an unequal number of subjects in each group. The number in each group is indicated in Fig. 2. Determinations of the size and accuracy of the lesion were made independently of any knowledge of the rats' behavioral data.
Behavioral data. The mean number of trials during which the rats in each group shuttle appeared in Fig. 2. A $2 \times 2 \times 2 \times 2$ (Lesion $\times$ Contiguity $\times$ Contingency $\times$ Session) ANOVA carried out on the shuttle data revealed that the lesioned rats shuttled significantly more than did the sham lesioned rats, $F(1,43) = 21.708$, $P < 0.0002$. Contiguity between tone and shock also significantly increased shuttling during the tone, $F(1,43) = 18.826$, $P < 0.0003$. The lesion effect was dependent on the presence of contiguity, with the VTA rats shuttling more than the controls only in those conditions in which tone and shock were paired, $F(1,43) = 16.008$, $P < 0.0005$.

Fig. 1. Maximum extent of the damage in the rats with the largest (shaded) and smallest (solid) lesions of the ventral tegmental area. VTA is outlined by the bold line. The drawing represents a section 4.8 mm posterior to Bregma, after the atlas of Paxinos and Watson (8). Abbreviations as follow: LG, lateral geniculate nucleus; MG, medial geniculate nucleus; SN, substantia nigra; MB, mammillary body; pc, posterior commisure; f, fornix; ml, medial lemniscus; cp, cerebral peduncle.

The response-shock contingency apparently was not as salient to the rats as was the tone-shock contiguity: the effect of contingency on shuttling failed to reach significance, $F(1,43) = 2.802$, $P < 0.10$. The effect of contiguity was slightly more pronounced in the contingent conditions, but this interaction also failed to reach significance, $F(1,43) = 2.862$, $P < 0.10$.

There was a significant effect of the session, $F(1,43) = 41.253$, $P < 0.0001$, indicating that the rats shuttled more in the second session than they did in the first. This factor interacted with the lesion, $F(1,43) = 7.649$, $P < 0.01$, with VTA rats improving more than shams. Session also interacted with contiguity, $F(1,43) = 11.658$, $P < 0.002$, indicating that improvement across sessions was greater in the conditions involving contiguous presentation of tone and shock than in the conditions involving unpaired presentation of tone and shock.
The lesion did not affect the general locomotor activity of the rats, as measured by the number of shuttle responses in the first 3 min in the chamber. The mean number of crossings made by the sham-lesioned rats was 13.09, while the VTA-lesioned rats made 13.72, $F(1,48) = 0.192$.

![Mean shuttle responses to tone](image)

**Fig. 2.** Mean ($\pm$ SEM) number of trials in which rats in each condition shuttle during tone presentation. In all four conditions involving tone-shock contiguity, VTA lesioned rats shuttled significantly more than the control rats. The number of rats in each group appears under each bar of the graph.

The latency to escape unavoidable shocks was reduced by the VTA lesion across all conditions: VTA mean = 0.98 s, Sham mean = 1.31 s, $F(1,43) = 9.972, P < 0.005$. The latency to escape was also reduced by contiguous presentation of tone and shock: contiguous mean = 1.03 s, noncontiguous mean = 1.26 s, $F(1,43) = 5.019, P < 0.03$, perhaps because conditioned responses that occurred late actually became very short latency escape responses in the contiguous conditions. There was no overall decrease in latency across the two sessions (Sessions 1 mean = 1.16 s, Session 2 mean = 1.13 s, $F(1,43) = 0.253$), but there was a significant
Session × Lesion × Contiguity interaction, $F_{(1,43)} = 4.679, P < 0.05$, that resulted from a decrease in escape latency among the slower learning sham-lesioned rats as the contiguity between tone and shock came to affect their behavior.

**DISCUSSION**

Shuttle responses controlled by the contiguity between tone and shock were facilitated by the lesion of the ventral tegmental area, while those controlled by the contingency between responding and shock avoidance were unaffected. These results indicate that the VTA normally inhibits those avoidance responses motivated by the classically conditioned fear of a warning signal. The general locomotor activity of the VTA- and sham-lesioned rats did not differ, indicating that the enhancement of shuttle responses by the lesion could not have resulted from a general activation of motor systems. The reduced latency to escape shock in the VTA lesioned rats reflects the hyperreactivity that typically accompanies that lesion (4). However, this hyperreactivity cannot account for increased shuttling in only two of the four conditions. We must conclude that the VTA lesion increases the probability of those shuttle responses motivated by classically conditioned fear.

Whether the VTA lesion disinhibits fear of the tone, or the responses motivated by that fear, cannot be determined in this experiment, as no assessment of fear independent of the shuttle response is available. However, in light of our previous research on the role of the nucleus accumbens in classically conditioned responses (11, 12), we suggest that the VTA-lesioned rats were not more fearful, but instead were more likely to act on their fear by shuttling. We have previously found that the infusion of dopamine into the nucleus accumbens attenuates classically conditioned motor responses, without affecting the sympathetic indices of fear recorded concurrently (12). This suggests that the removal of dopamine from the accumbens should facilitate classically conditioned motor responses, also without affecting conditioned sympathetic responses. One effect of the VTA lesion in the present experiment would have been to reduce dopamine levels in the accumbens, resulting in the predicted enhancement of classically conditioned motor responses. Confirmation of this interpretation would require further studies in which independent measures of fear are taken, and dopamine levels in the accumbens are assayed or manipulated directly. Nonetheless, the results concur with our previous findings, and further strengthen the hypothesis that dopamine in the accumbens acts normally to regulate motor responses motivated by fear.
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