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Effects of ventrolateral striatal inactivation on predatory hunting

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Abstract

Previous studies from our laboratory have shown that insect hunting is associated with a distinct Fos up-regulation in the ventrolateral caudoputamen at intermediate rostro-caudal levels. It is largely known that ventrolateral striatum participates in the control of orofacial movements and forepaw usage accompanying feeding behavior, but there has been no study investigating its possible roles during predatory hunting. We have presently examined the role of the ventrolateral striatum during roach hunting by using the reversible blockade with lidocaine. Accordingly, non-treated and saline-treated animals performed the insect hunting quite well, displaying a rather stereotyped form of motor actions for chasing, capturing and killing the prey. During the bilateral blockade of the ventrolateral striatum, the animals showed a significantly longer latency to start hunting and to capture the first prey. The lidocaine-treated animals captured the prey by using mostly the mouth, with little forepaw assistance, and were less effective in capturing the roaches. Moreover, while handling the prey, animals with ventrolateral striatal inactivation kept biting several parts of the prey, but failed to deliver the killing bite to the head, leaving them alive and moving, more likely to escape. Overall, the present findings suggest that the ventrolateral striatum implements the stereotyped actions seen during prey capture and handling, and may influence the motivational drive to start attacking the roaches, as well.

Keywords: Ventrolateral striatum; Predatory behavior; Feeding behavior; Lidocaine

1. Introduction

Predatory hunting has been regarded as an innate behavioral response seemingly critical for the animals' survival. Insect hunting has been proved to be a good model to study predatory activity in rats, which are known to display an innate pattern of prey hunting similar to the one seen in the small insectivores [1–3].

Previous studies from our laboratory have shown that insect hunting is associated with a distinct Fos up-regulation in the ventrolateral caudoputamen at intermediate rostro-caudal levels [3]. Given the stereotyped motor patterns seen in this behavior, and the fact that neostriatrum has been involved in organizing certain complex motor functions of behavioral sequencing [4], we have suggested that the ventrolateral striatum would be involved in organizing the stereotyped sequence of actions seen during insect hunting [3]. There is a wealth of data implicating the ventrolateral striatum in controlling orofacial movements and forepaw usage accompanying feeding behavior [5–9]. However, there has been no study investigating its possible roles during predatory hunting.

In the present study, we have examined the role of the ventrolateral striatum during roach hunting by using the reversible blockade with lidocaine. The reason for using this agent over more conventional permanent lesion techniques is that it provides temporary inactivation and does not present the compensatory neuronal effects that may arise during post-lesion recovery [10].

Overall, the present findings suggest that the ventrolateral striatum implements the stereotyped actions seen during prey

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capture and killing, and may influence the motivational drive to start attacking the roaches, as well.

2. Methods

Adult male Wistar rats ($n=36$), weighing about 250 g and obtained from the local breeding facilities, were used in the present study. The animals were kept under controlled temperature (23 °C) and illumination (12-h light/dark cycle) in the animal quarters, and had free access to water and standard laboratory diet (Nutrilab CR1; Nuvital Nutrientes, Ribeirão Preto, SP, Brazil). Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (1996). In the present study, we attempted to minimize the number of animals used and their suffering. One week before the experimental procedures, animals were individually housed, and were handled repeatedly by the same investigator, who conducted the behavioral tests. Animals were food deprived 24 h before the hunting sessions, which were carried out between 07:00 and 12:00 h, during the light phase of the cycle. Animals were initially submitted to a first session of hunting. First, the animals were individually transferred to a Plexiglas cage (50×35×16 cm), and after one-hour habituation period, induced to hunt by a simultaneous introduction, into the hunting cage, of five mature intact cockroaches (*Periplaneta americana*), raised for this purpose in our laboratory. The transfer to the hunting cage allowed for a clear view of the rat's behavior. The hunting behavior was videotaped for 30 min, and the following parameters were measured: latency to start hunting, latency to capture the first prey, number of unsuccessful attempts to capture the prey, and number of times a captured cockroach escaped from the rat.

Next, the rats were divided into control non-operated ($n=12$), saline ($n=12$) and lidocaine ($n=12$) groups. Animals from saline and lidocaine groups received bilateral implants of stainless-steel guide cannulas (23 gauge) aimed 2.0 mm above the ventrolateral striatum at the following coordinates: AP +9.0 mm from the interaural line; ML 3.5 mm from the midline; DV −5.5 mm from the skull. Animals were anesthetized with a mixture of ketamine (Vetaset; Fort Dodge Laboratory, Campinas, Brazil) and xylazine (Rompum; 1:2 v/v; 1 ml/kg body weight; Bayer, São Paulo, Brazil). Each cannula was fixed with polyacrylic cement anchored to the skull with stainless-steel screws and plugged with stainless-steel plugs.

One week after the surgery, animals were submitted to a second hunting session. As in the first one, animals had one-hour habituation period to the hunting cage. Immediately before the introduction of the cockroaches, animals from the saline and lidocaine groups received bilateral 0.4 μl injections of either saline (NaCL 0.9%) or 4% lidocaine (Sigma), respectively, into the ventrolateral striatum. For the injections, the animals were gently held and a removable injector was inserted into the guide cannula, extending 2 mm beyond the guide tip. The injector was linked to a 1 μl Hamilton syringe and the volume was injected over 1-min period. The injector was retained in place for an additional minute. Immediately afterward, five cockroaches were delivered into the hunting cage, and the animals were videotaped during 30 min for behavioral analysis. Rats of the non-operated control group underwent the second hunting session just like the first one, without any additional handling.

At the end of the experimental procedures, all rats were sacrificed with an overdose of pentobarbital. To check for cannula placement, the animals from the saline and lidocaine groups were transcardially perfused with saline solution followed by 10% formalin; the brains were immediately removed and post-fixed in the same fixative containing 20% sucrose. The brains were then frozen and 30 μm thick serial sections were cut in the frontal plane. The sections were mounted on gelatin-coated slides and stained with thionin. For the saline and lidocaine groups, only the animals with canulla placement into the ventrolateral striatum were included in the present analysis (saline group, $n=9$; lidocaine group, $n=10$).

2.1. Statistical analysis

The latencies to start hunting and to capture the first prey were analyzed by means of a parametric 3×2 two-way ANOVA, with Group (control, saline and lidocaine) and Session (first and second) as factors. Post-hoc analyses were performed by means of the Student–Newman–Keuls test. The number of unsuccessful captures and the number of prey escapes after capture were entered into a non-parametric analysis (Kruskal–Wallis ANOVA by ranks). Pairwise comparisons between groups were performed by means of the Mann–

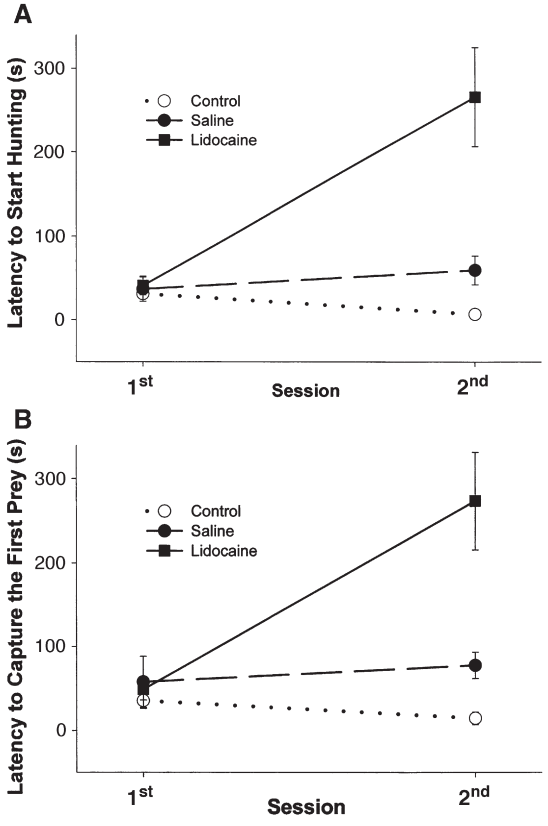

Fig. 1. Time (mean±SEM) taken to start hunting (A) and to capture (B) the first prey, measured from all experimental groups during the first and second hunting sessions.

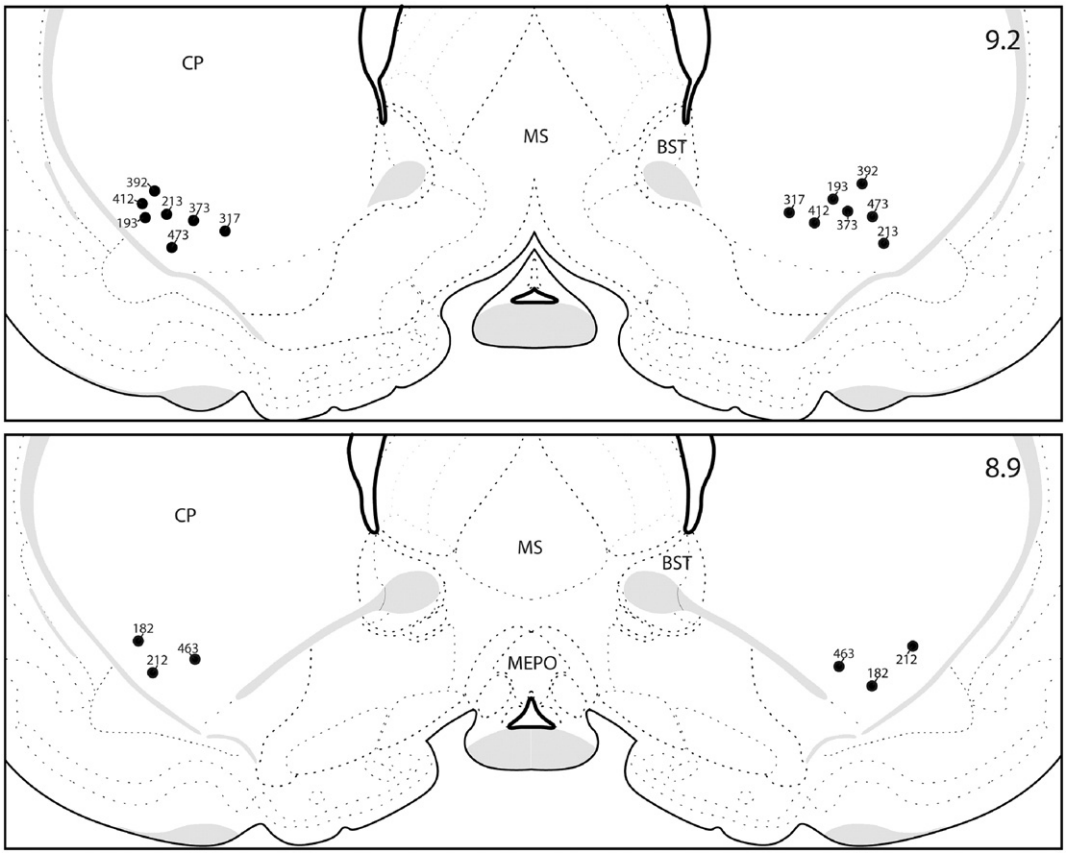

Fig. 2. Schematic representation of the bilateral injection sites in the ventrolateral striatum for the lidocaine-injected animals. The spots indicating the injection site placements are identified with the respective experiment numbers. The approximate distance from the interaural line is indicated on the upper right-hand corner of each figure. Abbreviations: BST — bed nuclei stria terminalis; CP — caudoputamen; MEPO — median preoptic nucleus; MS — medial septal nucleus.

Whitney *U* test [11]. The family-wise type I error was set at 5% for all statistical procedures.

3. Results

3.1. First hunting session

In this session, there was no difference among the experimental groups, and all animals presented similar predatory hunting pattern. By and large, animals started chasing the prey within the first minute after they had been delivered into the cage. At first, the rats rushed toward the roaches and tried to seize them. Both latencies to start hunting and to capture the first prey were measured (Fig. 1), and, in the first hunting session, they did not differ significantly among the experimental groups ($F_{2,28}<0.41$, $P>0.66$). Roach capture was done by catching the prey with the mouth and forepaws, and subsequently, firmly holding them with the forepaws. The killing bite was delivered shortly afterward, by

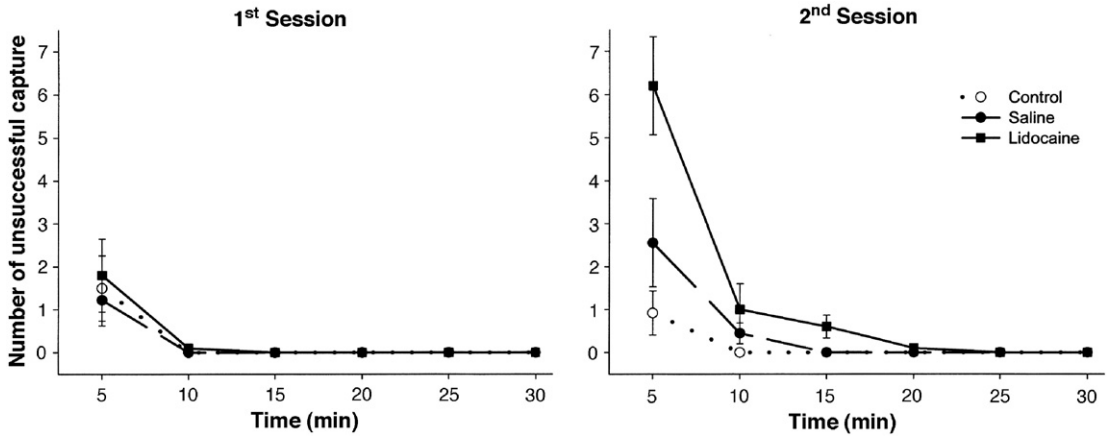

Fig. 3. Number of unsuccessful attempts to capture the prey (mean±SEM), tallied every 5 min during the first and second hunting sessions.

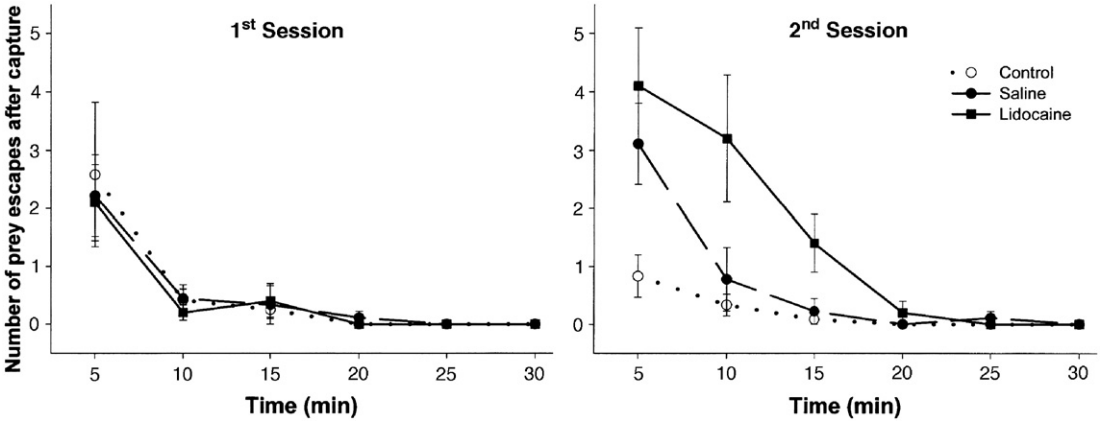

Fig. 4. Number of prey escapes after capture (mean±SEM), tallied every 5 min during the first and second hunting sessions.

ripping off the roaches' head. The killed roaches were usually taken to a corner of the cage and devoured voraciously. Although the tested animals had never hunted previously, they performed the insect hunting quite well, displaying a rather stereotyped form of motor actions for chasing, capturing and killing the prey.

3.2. Second hunting session

Compared to other experimental groups, lidocaine-treated animals presented clear deficits in insect hunting, which were particularly seen during the first 10 min of the observation period (see Fig. 2 for injection site placements). Both the untreated and saline-treated groups behaved similarly to what we have just previously described, and particularly the animals from the untreated group showed some improvement in the predatory parameters tested during the second hunting session. Lidocaine-treated animals showed a significantly longer latency (Fig. 1) to start hunting ($P<0.01$) and to capture the first prey ($P<0.01$). At the beginning, by the time the roaches had been delivered into the cage, the lidocaine-treated animals sniffed them and wandered around the cage, without attacking the roaches. In sharp contrast to the other groups, during the capture, the lidocaine-treated animals used mostly the mouth, with little forepaw assistance; as a consequence, they had a significantly larger number of unsuccessful approaches as they tried to capture the prey ($P<0.01$, Fig. 3). Once the lidocaine-treated animals captured the prey, they held them less firmly, and failed to immediately deliver the killing bite to the head, but instead, bit other regions of the prey's body, leaving the roaches alive and moving for longer periods, and therefore, more likely to escape ($P<0.01$, Fig. 4). In short, lidocaine injection into the ventrolateral striatum induced a significant delay in starting to chase the prey, as well as apparent deficits in capturing, holding and killing the prey, influencing, perhaps, the stereotyped sequence of actions seen during insect hunting. Curiously, saline-tested animals, at the beginning of the second hunting session, immediately after they had received the saline injection, also had some difficulty in holding the prey ($P=0.01$, Fig. 4).

4. Discussion

The present results suggest a role for the ventrolateral striatum in organizing the predatory behavior motor output, and, perhaps, in influencing the motivational drive to hunt the prey, as well.

To investigate the ventrolateral striatal putative roles during predatory hunting, we used lidocaine temporary neuronal blockade, to avoid any possible compensatory neuronal effects that could arise in response to permanent lesions. Previous studies have estimated neuronal inactivation time course following injections of lidocaine, and found that the blocking effect peaks around 10 min, reduces significantly during the following 15 min and are largely over by 30 min [12]. In line with this view, the effects of the ventrolateral striatal inactivation seen in the present investigation were particularly noticeable within the first 10 min after the injection. This short inactivation period left a relatively narrow window to observe the behavioral changes due to the ventrolateral striatal blockade, and certainly represented a limitation to the present analysis. With this caveat in mind, we shall now discuss the main changes seen during insect hunting in response to the ventrolateral striatal inactivation.

Lidocaine-treated animals presented a significantly longer latency to start hunting the prey. During the peak of the lidocaine-induced ventrolateral striatal inactivation, the animals did not present any clear motor deficit and kept exploring the cage and sniffing the roaches, showing relatively little interest in chasing the prey. This finding is similar to what we had previously found for animals with lateral periaquedutal gray lesions [13] and suggests that the ventrolateral striatum may be involved in modulating the motivational drive to hunt. In fact, a number of studies revealed a potential role for the ventrolateral striatum in reward mechanisms. It has been shown that opioid stimulation in the ventrolateral striatum enhances the intake of palatable food [14], and injection of low amphetamine doses into this striatal region stimulates feeding in satiated animals [15] and produces conditioned place preference [16].

In agreement with previous studies, we have presently seen that animals hunting for the first time already present a stereotyped sequence of actions, supporting the idea of an innate motor program to capture and handle the prey, which certainly increases hunting efficiency [3]. Ventrolateral striatal inactivation does not produce any apparent motor impairment, but seems to interfere with the innate ability to capture and handle the prey. Accordingly, ventrolateral striatal inactivation renders capture procedures less

efficient, and the animals try to seize the prey mostly using the mouth, with little assistance from the forepaws. Moreover, while handling the prey, animals with ventrolateral striatal inactivation kept biting several parts of the prey, but failed to deliver the killing bite to the head, leaving them alive and moving, more likely to escape. It is noteworthy that saline injection induced a very short lasting prey handling deficiency, probably due to a transient structural disruption caused by the tissue accommodation of the small saline volume injected.

There is a wealth of experimental evidence suggesting the ventrolateral striatum as critically involved in controlling orofacial and forepaw motor function [17,18]. Of particular relevance for the present context, however, the striatum may be involved in organizing certain complex motor functions of behavioral sequencing, such as grooming [4]. In this sense, considering our results, it seems reasonable to believe that the ventrolateral striatum would be involved in implementing the sequential pattern of action during predatory hunting.

In short, the use of the short-lasting lidocaine inactivation helped us to reveal that the ventrolateral striatum is a likely candidate to implement the stereotyped sequence of actions seen during prey capture and handling, and perhaps to influence the motivational drive to hunt. However, the relatively short period of lidocaine effect seen in our experiments may have posed some constraints to fully appreciate the behavioral consequences related to ventrolateral striatal inactivation, and additional studies using long lasting inactivation, or even more permanent lesion methods, are likely to improve the evaluation of our hypothesis.

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