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THE ROLE OF THE SUPERIOR COLLICULUS IN PREDATORY HUNTING

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Abstract—Combining the results of behavioral, neuronal immediate early gene activation, lesion and neuroanatomical experiments, we have presently investigated the role of the superior colliculus (SC) in predatory hunting. First, we have shown that insect hunting is associated with a characteristic large increase in Fos expression in the lateral part of the intermediate gray layer of the SC (SCig). Next, we have shown that animals with bilateral NMDA lesions of the lateral parts of the SC presented a significant delay in starting to chase the prey and longer periods engaged in other activities than predatory hunting. They also showed a clear deficit to orient themselves toward the moving prey and lost the stereotyped sequence of actions seen for capturing, holding and killing the prey. Our *Phaseolus vulgaris*-leucoagglutinin analysis revealed that the lateral SCig, besides providing the well-documented descending crossed pathway to premotor sites in brainstem and spinal cord, projects to a number of midbrain and diencephalic sites likely to influence key functions in the context of the predatory behavior, such as general levels of arousal, motivational level to hunt or forage, behavioral planning, appropriate selection of the basal ganglia motor plan to hunt, and motor output of the primary motor cortex. In contrast to the lateral SC lesions, medial SC lesions produced a small deficit in predatory hunting, and compared to what we have seen for the lateral SCig, the medial SCig has a very limited set of projections to thalamic sites related to the control of motor planning or motor output, and provides conspicuous inputs to brainstem sites involved in organizing a wide range of anti-predatory defensive responses. Overall, the present results served to clarify how the different functional domains in the SC may mediate the decision to pursue and hunt a prey or escape from a predator. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: aggression, motor control, basal ganglia, thalamus, periaqueductal gray, defensive behavior.

The superior colliculus (SC) is a midbrain structure known to be important for controlling spatial orienting when guided by visual, auditory, somatosensory and even olfactory cues (Grobstein, 1988; Dean et al., 1989; King, 2004; Boehnke and Munoz, 2008; Felsen and Mainen, 2008), and is likely to exert a key role in prey hunting. On the sensory side, the lateral SC may integrate critical sensory information to prey detection, such as small dark moving objects in the lower rostral and lateral visual field (Dean et al., 1989); and on the motor side, studies using either electrical or pharmacological stimulation have shown that the lateral SC may elicit a wide range of contralaterally directed movements, which may mimic pursuit of a moving object (Dean et al., 1986, 1988; Sahibzada et al., 1986). In line with this view, it has been shown that, following tectal undercuts, hamsters presented impaired reorientation to and pursuit of crickets jumping out of the grasp into the visual periphery (Finlay et al., 1980). However, a more comprehensive analysis on how the SC would be involved in controlling hunting behavior is still missing. Therefore, in the present study, combining the results of behavioral, neuronal immediate early gene activation, lesion and neuroanatomical experiments, we have investigated the role of the SC in predatory hunting.

To study predatory behavior, we have used insect hunting, which appears as an ideal condition to investigate predatory behavior in rats (Comoli et al., 2005). In this paradigm, roaches have been chosen as suitable prey, since they are relatively innocuous and easily overcome. In addition, considering the voracity that the rats present to consume the roaches, they are supposedly very palatable, with potentially high hedonic value.

In the present investigation, we have first determined the pattern of Fos expression in the SC of animals that had preformed insect hunting, and next, examined how bilat-

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Abbreviations: APN, anterior pretectal nucleus; CL, central lateral nucleus thalamus; CM, central medial nucleus thalamus; CUN, cuneiform nucleus; fr, fasciculus retroflexus; III, oculomotor nucleus; ICe, inferior colliculus, external nucleus; LD, lateral dorsal nucleus thalamus; LGd, lateral geniculate complex, dorsal part; LGv, lateral geniculate complex, ventral part; LH, lateral habenula; LP, lateral posterior nucleus thalamus; MDl, mediodorsal nucleus thalamus, lateral part; MDm, mediodorsal nucleus thalamus, medial part; MH, medial habenula; MO, somatomotor cortex; MPT, medial pretectal area; MRN, midbrain reticular nucleus; OP, olivary pretectal nucleus; PAG, periaqueductal gray; PAGdl, periaqueductal gray, dorsolateral division; PAGdm, periaqueductal gray, dorsomedial division; PAGl, periaqueductal gray, lateral division; PAGvl, periaqueductal gray, ventrolateral division; PBG, parabigeminal nucleus; PCN, paracentral nucleus thalamus; PF, parafascicular nucleus thalamus; PFC, prefrontal cortex; PO, posterior complex thalamus; PRC, precomissural nucleus; RE, nucleus reuniens; RH, rhomboid nucleus; SAG, nucleus sagulum; SC, superior colliculus; SCdg, superior colliculus, deep gray layer; SCig, superior colliculus, intermediate gray layer; SCsg, superior colliculus, superficial gray layer; SNc, Substantia nigra, compact part; SNr, Substantia nigra, reticular part; SOM, supraoculomotor area; SS, somatosensory cortex; VAL, ventral anterior-lateral complex thalamus; VL striatum, vetrolateral striatum; VM, ventral medial nucleus thalamus; VP, ventral posterior complex thalamus; ZI, zona incerta.

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eral lesions placed in the medial or lateral parts of the SC would interfere with the hunting performance. The study was complemented with a *Phaseolus vulgaris*-leucoagglutinin (PHA-L) tract-tracing analysis, which provided an interesting perspective on how the diverse SC projections may influence a wide range of functions related to hunting behavior. The present investigation presents an important piece of information that expands our previous analysis on the neural systems underlying predatory hunting (Comoli et al., 2003b, 2005; Sukikara et al., 2006; dos Santos et al., 2007) and helps to clarify how the different functional domains in the SC may mediate the decision to pursue or escape from a given stimulus.

EXPERIMENTAL PROCEDURES

Animals and housing

Adult male Wistar rats ($n=48$), weighing about 250 g and obtained from the local São Paulo breeding facilities, were used in the present study. The animals were kept under controlled temperature (23 °C) and illumination (12 h cycle) in the animal quarters, and had free access to water and standard laboratory diet. Experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996). All experimental procedures had been previously approved by the Committee on Care and Use of Laboratory Animals of the Institute of Biomedical Sciences—University of São Paulo, Brazil (Protocol number 084/2005). In the present study, we attempted to minimize the number of animals used and their suffering.

Experimental apparatus and procedure

One week before the experimental procedures, animals were individually housed into a plexiglas cage ($50\times35\times16$ cm^3), and were handled repeatedly by the same investigator who had conducted the behavioral tests. Animals were food deprived 24 h before the hunting sessions, which were carried out between 9:00 and 12:00 h, during the light phase of the cycle. In the hunting session, animals were 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 hunting behavior was videotaped for further behavioral analysis.

Behavior analysis

Behaviors were scored by a trained observer using the ethological analysis software "The Observer" (version 5.0; Noldus Information Technology, Wageningen, The Netherlands). For the behavioral analysis of predatory hunting, we have first determined the latency to start hunting, and in the following 15 min, we have carefully examined the motor pattern to capture, hold and kill the prey, and recorded the following behavioral parameters: the number of attempts to capture the prey, number of successful captures (when the animals could hold the prey for more than 10 s), time spent eating, time spent displaying other behaviors than hunting (i.e., grooming, general exploratory activity and resting).

Experiment 1

In experiment 1, we have analyzed the Fos expression in the SC of animals that had displayed predatory hunting ($n=5$) and compared to control animals ($n=5$) that were housed and handled in the same way as the animals that had performed insect predation, but were left undisturbed in the cage during the test period.

Ninety minutes after the testing procedures, animals were deeply anesthetized with sodium pentobarbital (Cristália, Itapira, SP, Brazil; 40 mg/kg i.p.) and perfused transcardially with a solution of 4.0% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4; the brains were removed and left overnight in a solution of 20% sucrose in 0.1 M phosphate buffer at 4 °C. The brains were then frozen and four series of 30 μm-thick sections were cut with a sliding microtome in the frontal/transverse plane. One series was processed for immunohistochemistry with anti-Fos antiserum raised in rabbit (Ab-5, Calbiochem, San Diego, CA, USA; lot # D09803) at a dilution of 1:20,000. The primary antiserum was localized using a variation of the avidin–biotin complex system. In brief, sections were incubated for 90 min at room temperature in a solution of biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA), and then placed in the mixed avidin–biotin horseradish peroxidase (HRP) complex solution (ABC Elite Kit; Vector Laboratories) for the same period of time. The peroxidase complex was visualized by a 10 min exposure to a chromogen solution containing 0.02% 3,3′diaminobenzidine tetrahydrochloride (DAB, Sigma, St Louis, MO, USA) with 0.3% nickel-ammonium sulfate in 0.05 M Tris–buffer (pH 7.6), followed by incubation for 10 min, in chromogen solution with hydrogen peroxide (1:3000) to produce a blue-black product. The reaction was stopped by extensive washing in potassium phosphate-buffered saline (KPBS; pH 7.4). Sections were mounted on gelatin-coated slides, and then dehydrated and coverslipped with DPX (Sigma). An adjacent series was always stained with Thionin to serve as a reference series for cytoarchitectonic purposes.

Counts of the number of Fos-immunoreactive neurons were evaluated by an observer without knowledge of the animal's experimental status and were generated for the SC at its mid rostrocaudal levels, where the SC presents its largest size. Sections were viewed under a 20× objective of a Nikon Eclipse E600 microscope equipped with a camera lucida, and an optical graticule was used to manually quantify the number of Fos-positive neurons in the lateral, intermediate, and medial parts of the superior colliculus (Fig. 1A). Only darkly labeled oval-shaped nuclei that fell within the grid were counted. The number of positive nuclei that fell within a 0.3×0.3 mm^2 area (0.09 mm^2) in each region of interest was counted from one section per rat (Fig. 1A).

Experiment 2

In experiment 2, we examined the behavioral changes observed in the hunting behavior of animals with bilateral lesions in the lateral part of the SC, and compared with those with bilateral lesions in the medial part of the SC and sham-lesioned animals.

For the lesion procedure, rats were deeply anesthetized with sodium pentobarbital (40 mg/kg i.p.) and were placed in a stereotaxic apparatus. Bilateral iontophoretic deposits of a 0.15 M solution of N-methyl-D-aspartate (NMDA, Sigma, St. Louis, MO, USA) were placed in the lateral part of the SC of 10 animals and in the medial part of the SC of 10 animals, in addition to control saline injections that were made in other 10 rats. NMDA deposits were produced over 15 min through a glass micropipette (30 μm tip diameter), using a constant-current device (model CS3, Midgard Electronics, Canton, MA, USA) set to deliver -10 μA, with 7-s pulse and interpulse durations. Animals recovered for 2 weeks after surgery, before the predatory hunting test session.

Experiment 3

In experiment 3, we have investigated the projections of the medial and the lateral parts of the intermediate gray layer of the SC (SCig). Eight animals received a single injection of *Phaseolus vulgaris*-leucoagglutinin (PHA-L, Vector, Burlingame, CA, USA) into the SC (four animals in the lateral SCig and four animals in the medial SCig). First, they were anesthetized with a mixture of ketamine and xylazine (v/v; 1 ml/kg body weight), and then the iontophoretic injection of a 2.5% solution of PHA-L in 0.1 M sodium phosphate-buffered saline, pH 7.4 (35) was made over 10 min through a stereotaxically positioned glass micropipette (10 μm tip diameter) by applying a $+5$ μA current, pulsed at 7 s