

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 mid-brain 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-

¹ ICF and WFO had the same participation in the study.

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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; MDI, mediadorsal nucleus thalamus, lateral part; MDm, mediadorsal 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, precommissural 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, ventrolateral striatum; VM, ventral medial nucleus thalamus; VP, ventral posterior complex thalamus; ZI, zona incerta.

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 ($50 \times 35 \times 16$ cm³), 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 μ m-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 \times 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 mm² area (0.09 mm²) 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 μ m tip diameter), using a constant-current device (model CS3, Midgard Electronics, Canton, MA, USA) set to deliver -10 μ A, 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 μ m tip diameter) by applying a +5 μ A current, pulsed at 7 s

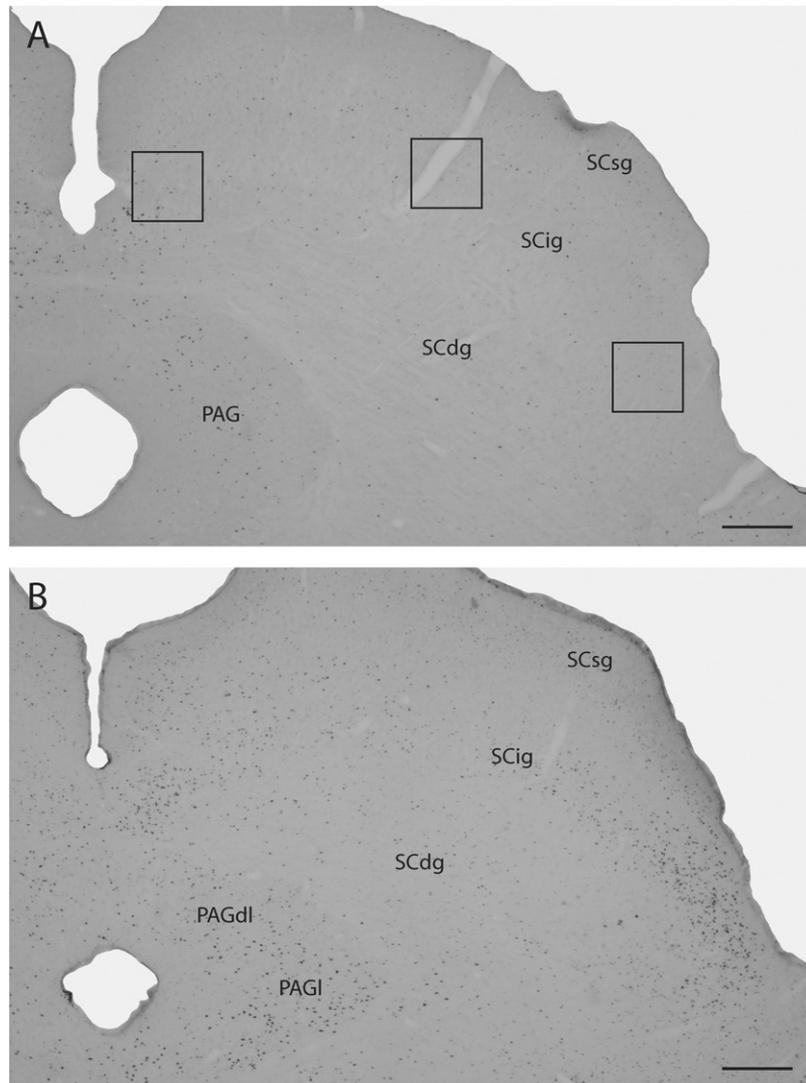


Fig. 1. Experiment 1—Photomicrographs of transverse Fos-stained sections of the SC from a control animal (A) and a rat that had performed predatory hunting (B). Open squares indicate the position of the 0.09 mm² grid (exactly to scale) within which the Fos counts were made. Abbreviations: see list. Scale bars=300 μ m.

intervals, with a constant-current source (Midgard Electronics, Wood Dale, IL, USA, model CS3). After a survival time of 14–16 day, the animals were perfused, and the brains processed as described for Fos immunohistochemistry. One series of sections was processed for immunohistochemistry with an antiserum directed against PHA-L (Dako, Carpinteria, CA, USA) at a dilution of 1:5000, and the antigen–antibody complex was localized following the procedure described for Fos immunohistochemistry. The sections were mounted on gelatin-coated slides and then treated with osmium tetroxide to enhance visibility of the reaction product. Slides were then dehydrated and coverslipped with DPX. An adjacent series was always stained with Thionin to serve as a reference for cytoarchitecture.

The figures were prepared for publication by using the Adobe Photoshop (version 4.0; Adobe Systems, Mountain View, CA, USA) for photomicrographs and Adobe Illustrator (version 10.0; Adobe Systems) for line drawings. Only sharpness, contrast, and brightness were adjusted. Unless otherwise indicated, parcellation of the brain regions follows Swanson (1992).

Statistical analysis

After having tested for homogeneity of variances, the data on the density of Fos labeled cells were analyzed using a parametric analysis of variance (ANOVA), followed by planned pair wise comparisons. In order to keep the overall type I error at 5%, the significance level employed was adjusted downward according to Bonferroni correction ($\alpha=0.017$).

In experiment 2, for behavioral measurements, a nonparametric method, the Kruskal–Wallis test, was used due to severe violations of the homogeneity of variances assumption by the recorded data. The significance level was set at $\alpha=5\%$. Average results are expressed as mean \pm SEM throughout the text.

RESULTS

Experiment 1

In this experiment, we have examined the pattern of Fos expression in the SC of animals that had performed predatory hunting. The group of animals exposed to the

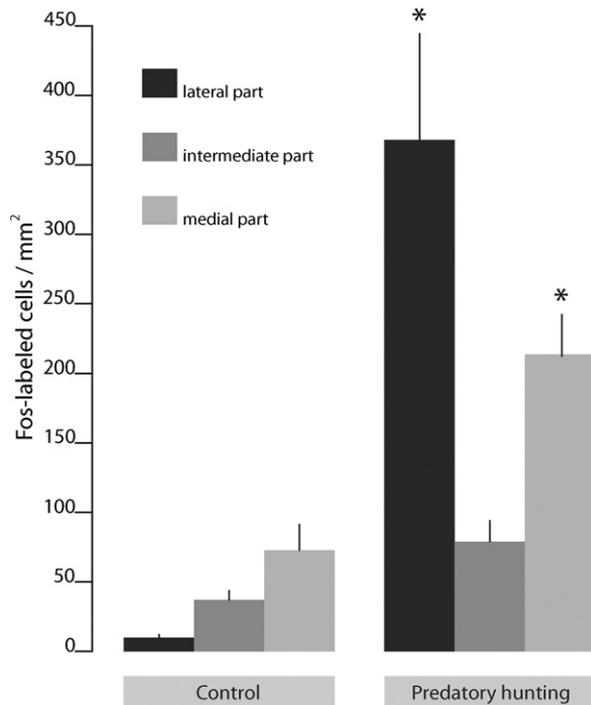


Fig. 2. Experiment 1—Frequency histograms show the density of Fos-immunoreactive cells in the lateral, intermediate, and medial parts of the SC in control animals ($n=5$) and animals that had performed predatory hunting ($n=5$). Values represent the number of Fos-labeled cells/mm². Data are expressed as mean \pm SEM. * Differs significantly compared to the same region in the control group, $P<0.001$.

roaches presented the characteristic insect hunting behavioral pattern. They started chasing the prey immediately after they were placed in the testing box, and captured

them efficiently with the mouths and forepaws. Subsequently, the rats firmly held the prey, delivered the killing bite, ripping off the roaches' head, and started eating them voraciously. Compared to the control group (animals not exposed to the roaches), the rats that displayed predatory hunting presented a significant increase in Fos levels in the SC ($F_{2,34}=15.701$; $P=0.00001$). Planned pair wise comparisons revealed that predatory hunting was associated with a significant Fos increase both in the lateral ($P=0.001$) and medial ($P=0.001$) regions of the SC (Fig. 2). In contrast, Fos expression in the intermediate collicular regions did not differ between the studied groups ($P=0.025$). As seen in Fig. 1, in animals that performed predatory hunting, the increase in Fos expression was particularly prominent in the lateral SC, where Fos-labeled cells tended to be mostly distributed in the superficial regions of the intermediate gray layer of the SC (layer a and superficial layer b).

Experiment 2

In experiment 2, intact animals and animals with NMDA lesions in the lateral or medial parts of the SC were tested during insect hunting. The parameters described above for NMDA iontophoretic injections resulted in relatively circumscribed lesions, characterized by neuronal cell loss filled with gliosis (Fig. 3). In six animals, the lesions were centered bilaterally in the lateral part of the SC (SCI), encompassing the superficial, intermediate and, to a lesser extent, deep layers of the SC, extending through the intermediate two-thirds of the rostrocaudal SC axis (Figs. 3A and 4). In five animals, lesions bilaterally hit all layers of the medial part of the SC (SCm), chiefly covering the intermediate two-thirds of the rostrocaudal SC extent (Figs. 3B and 4).

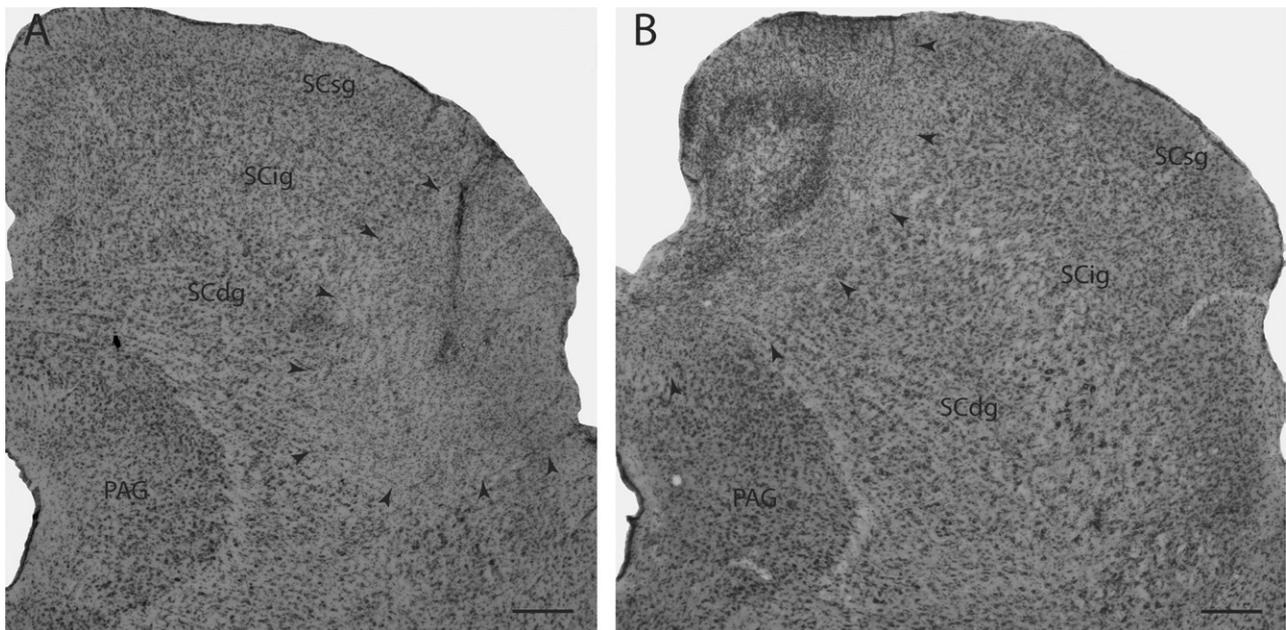


Fig. 3. Experiment 2—NMDA lesion appearance. Photomicrographs of transverse thionin-stained sections illustrating the extent and appearance of lesions in the lateral (A) and medial (B) parts of the SC (arrowheads), from representative cases in experiment 2. Abbreviations: see list. Scale bars=300 μ m.

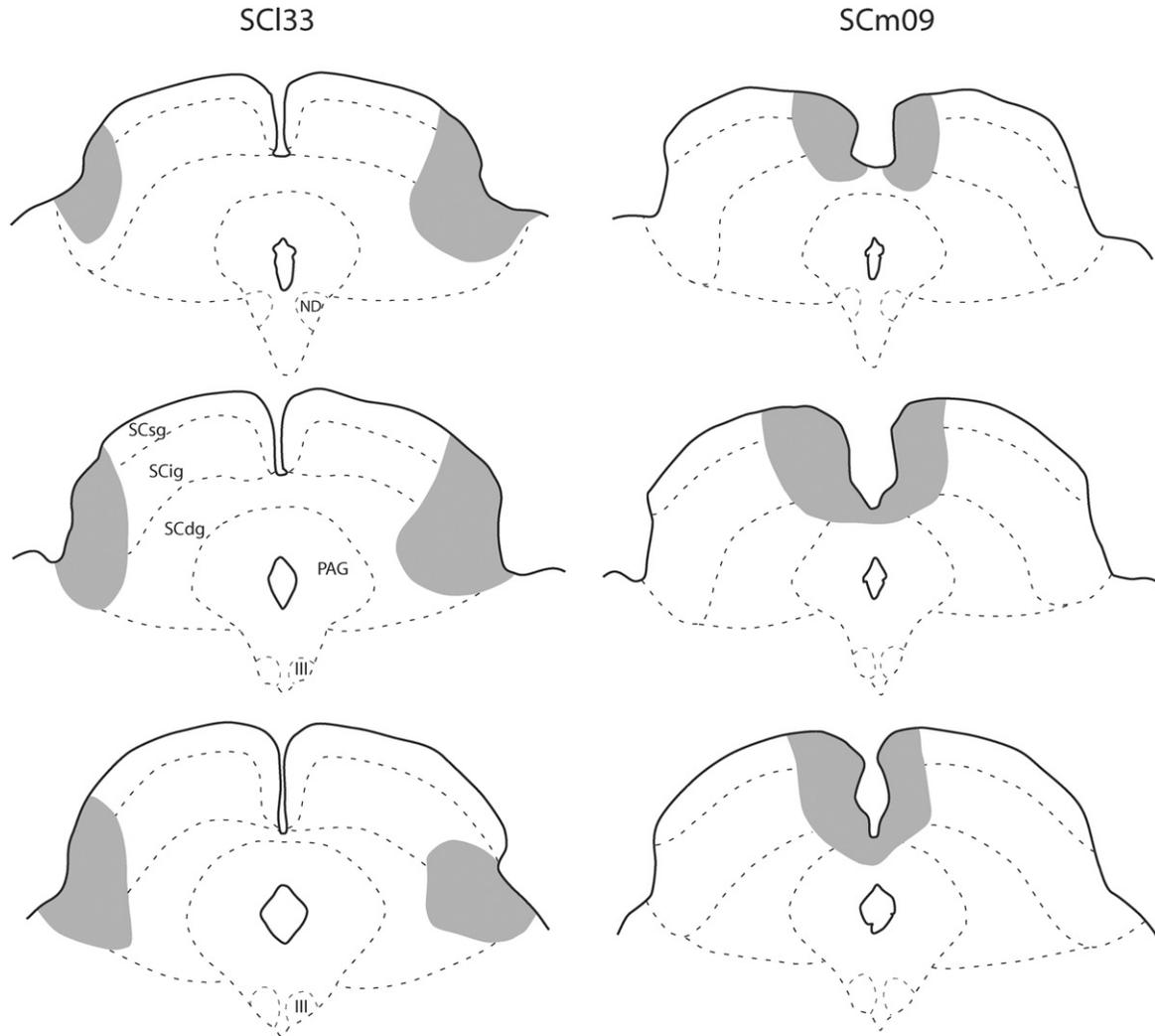


Fig. 4. Experiment 2—Bilateral SC lesions. Camera lucida drawings from representative cases in experiment 2 show the total extent of bilateral lesions in the lateral (A, experiment SCI33) and medial (B, experiment SCm09) parts of the SC. Sections arranged from rostral to caudal (top to bottom). Abbreviations: see list.

Behavioral analysis revealed that sham-lesioned animals started chasing the prey shortly after they had been delivered into the testing box (10.0 ± 2.6 s), orienting themselves very efficiently toward the moving prey, while trying to capture them. The capture was performed using the mouth, assisted by the forepaws. These animals caught the prey very efficiently, presenting a close to one ratio between the number of successful captures and the total number of catching attempts (Fig. 5B). As the prey had been captured, animals held them firmly with the forepaws and delivered the killing bite, ripping off the roaches' head. After killing the prey, animals either started eating them right away or carried on hunting other prey to consume them afterwards. The rats usually took the killed roaches to a corner of the cage and tried to conceal the captured prey from other potential predators (dodging behavior) while eating them voraciously. It is noteworthy that these animals did not have a previous hunting experience, but performed the insect hunting quite well, displaying a rather

stereotyped sequence of motor actions for chasing, capturing and killing the prey.

Bilateral SCI lesions produced multiple deficits during prey hunting. First, SCI-lesioned rats presented a clear delay in starting to engage in predatory hunting, and, compared to intact animals, showed a significantly longer latency to start chasing the roaches placed in the testing box (Fig. 5A, $P=0.003$). During chasing, SCI-lesioned animals frequently lost track of the roaches, and failed to orient themselves toward the moving prey. They were also very ineffective when trying to catch the roaches. First, they tried to seize them usually with the forepaws, but repeatedly let them escape. To hold the prey, they used mostly the mouth, with little assistance of the forepaws. Therefore, the prey capture was very ineffective, and, compared to intact rats, SCI-lesioned animals presented a significant drop in the ratio of successful captures (Fig. 5B, $P=0.001$). Compared to the other groups, SCI-lesioned animals also looked very clumsy when trying to hold the prey, and failed

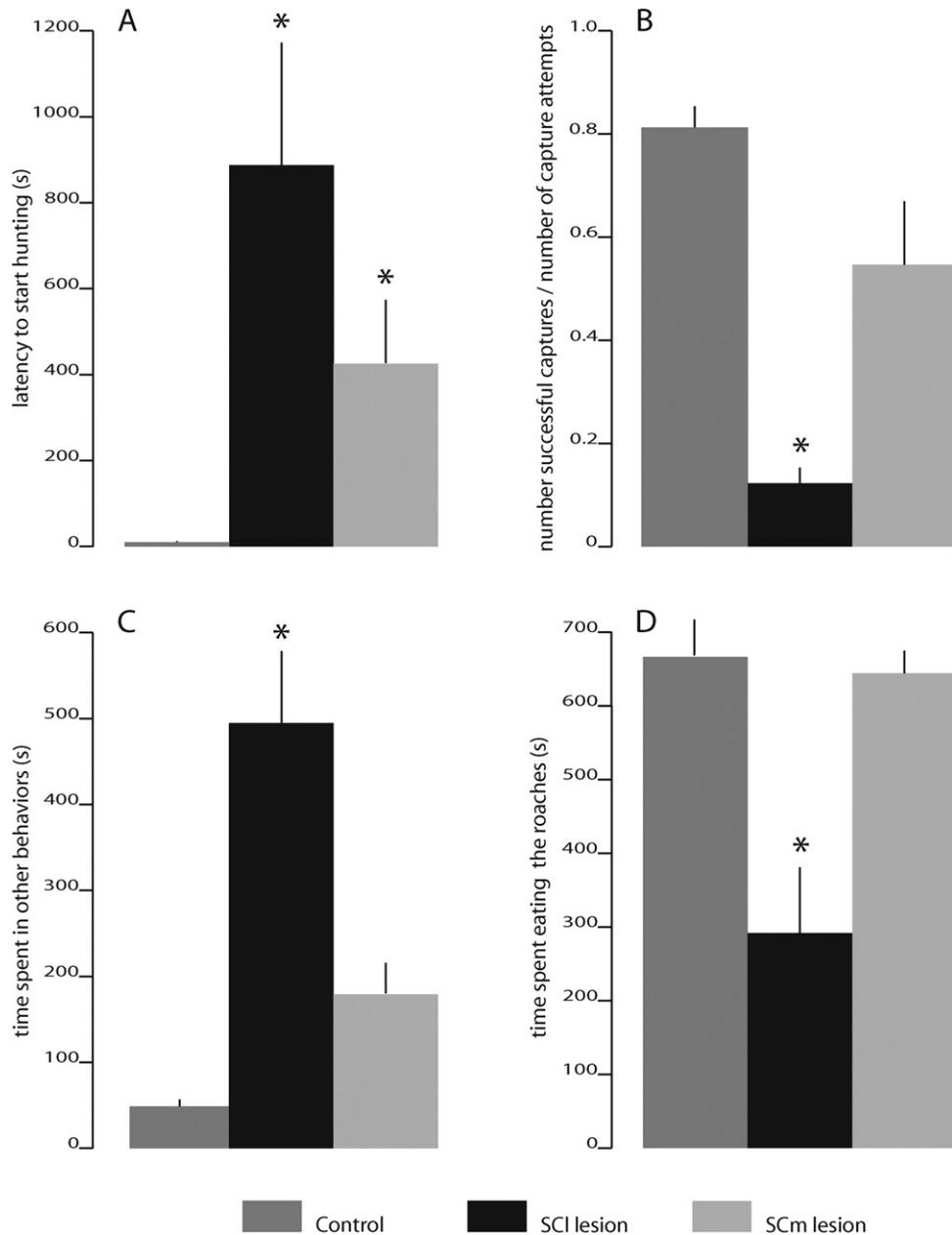


Fig. 5. Experiment 2—Behavioral analysis. The behavioral analysis of experiment 2 shows the latency to start hunting (A), the ratio between the number of successful captures and number of capture attempts (B), time spent in other behaviors (C), and time spent eating (D); for the sham-lesioned animals (control; $n=8$); the animals with bilateral lesions in the lateral SC (SCI-lesion; $n=6$); and the animals with bilateral lesions in the medial SC (SCm-lesion; $n=5$). Data are expressed as mean \pm SEM. * Differs significantly from the control group, $P < 0.05$.

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. Moreover, compared to sham-lesioned animals, SCI-lesioned rats were less engaged in the predatory hunting, and spent significantly more time displaying other behaviors, such as grooming, general exploratory activity and resting (Fig. 5C, $P=0.0004$). In short, SCI-lesioned rats 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.

In sharp contrast to SCI lesions, SCm lesions produced a small deficit in predatory hunting. SCm-lesioned animals also presented a certain delay to start hunting, representing the only insect hunting parameter recorded that significantly differed from the sham-lesioned animals (Fig. 5A, $P=0.026$). Similar to the intact animals, however, they were able to orient themselves toward the moving prey and perform the chasing without losing track of them. As the

intact animals, SCm lesioned rats presented a clear stereotyped sequence of actions, as already described for capturing, holding and killing the prey. They seized the prey with the forepaws and mouth, and subsequently held them with the forepaws to deliver the killing bite, ripping off the roaches' head. Apparently, SCm-lesioned animals seem to hold the prey less firmly, rendering them more likely to escape. SCm-lesioned animals presented a decrease in the ratio of successful captures, which, however, did not differ significantly from the intact animals (Fig. 5B, $P=0.683$).

Experiment 3—Projections of the SC

Taken together, the results of experiment 1 and 2 support the idea that the lateral part of the intermediate gray layer of the SC (lateral SCig) is a critical element in the control of predatory hunting. Therefore, to understand the potential paths involved in this control, in experiment 3, we have revisited the efferent connections of lateral SCig, and further compared these results with those obtained on the projections of the medial part of the intermediate gray layer of the SC (medial SCig), which belongs to a different functional domain of the SC.

Projections of the lateral part of the SCig. In three experiments, the PHA-L injections labeled neurons that were mostly confined to the lateral part of the SCig, coinciding with the SC region particularly activated during predatory hunting (see experiment 1) and the region where NMDA lesions resulted in a major deficit in the hunting performance (see experiment 2). In all of these experiments, a very similar pattern of anterogradely labeled fibers was observed, and of these, we chose experiment PHA-LSC#4 as a prototype to illustrate our results, because the injection in that experiment labeled the most extensive population of cells in the lateral SCig (Fig. 6A).

From the injection site, projecting fibers first provide a dense terminal field in the adjacent deep gray layer of the SC. At the injection site levels, labeled fibers and terminals

could also be traced to a number of midbrain sites, including the periaqueductal gray, the mesencephalic reticular nucleus, and the compact part of substantia nigra. Of particular relevance, the projection to the periaqueductal gray was particularly aimed at the lateral column at the level of the oculomotor nucleus, and to a lesser degree, to the supraoculomotor region, as well (Fig. 7). From the injection site levels, projecting fibers may either follow descending routes to caudal midbrain, pons and medulla, or ascending paths to the pretectum, diencephalon and telencephalon.

Our observations on the descending projections to the brainstem are in full agreement with previous studies (Chevalier and Deniau, 1984; Redgrave et al., 1986, 1987; Bickford and Hall, 1989), and will be briefly described here. Descending fibers from the lateral SCig may be divided into a relatively small ipsilateral route and a more robust contralateral path following the tectospinal path, also known as the predorsal bundle. Descending fibers following the ipsilateral path provide relatively sparse projections to the cuneiform and pedunculo-pontine nuclei, and lateral aspects of the pontine reticular nucleus. The more prominent contralateral contingent of descending fibers follows the predorsal bundle, and, at pontine levels, provides a moderate projection to the paramedian region of the pontine reticular nucleus, in addition to a relatively dense terminal field to medial aspects of the tegmental reticular nucleus. At these levels, a small contingent of these descending fibers also takes a dorsolateral course to project to the pedunculo-pontine nucleus, and a few of these labeled axons may extend further dorsally to reach the laterodorsal tegmental nucleus. Proceeding caudally, at medullary levels, fibers coursing through the tectospinal path provide a widespread projection to the gigantocellular and medullary reticular nuclei, as well as the parvocellular reticular nucleus. At rostral medullary levels, a prominent number of fibers and terminals could be found in the region of the gigantocellular reticular nucleus close to the abdu-

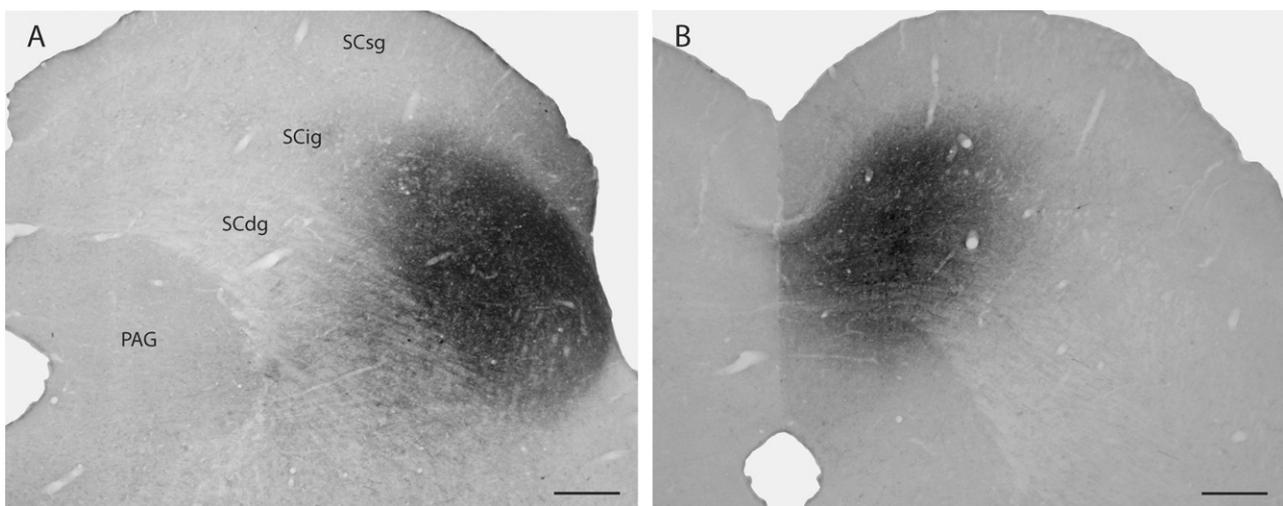


Fig. 6. Experiment 3—Brightfield photomicrographs showing PHA-L deposits in the lateral part (A, experiment PHA-LSC#4) and in the medial part (B, experiment PHA-LSC#7) of the intermediate gray layer of the SC. Abbreviations: see list. Scale bars=300 μ m.

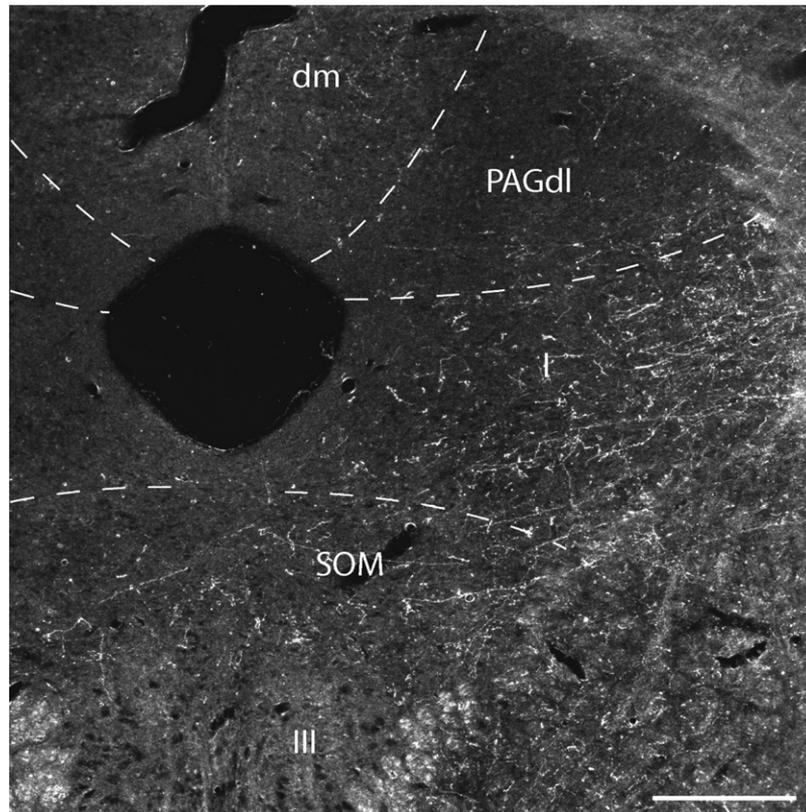


Fig. 7. Experiment 3—Darkfield photomicrograph showing the distribution of PHA-L-labeled axons at rostral levels of the periaqueductal gray (experiment PHA-LSC#4 with a PHA-L injection centered in the lateral part of the intermediate gray layer of the SC). Abbreviations: see list. Scale bar=300 μm .

cens nucleus, in the periaqueductal area. At caudal medullary levels, we have also found that descending fibers following the tectospinal path provide a relatively dense terminal field in the medial accessory olive. In the present experiments, the spinal cord projections were not examined.

Our PHA-L experiments also revealed that the lateral SCig provides quite substantial ascending projections to the pretectum and diencephalon. In the pretectum, the lateral SCig projects densely to the anterior pretectal nucleus. Proceeding rostrally, ascending fibers from the lateral SCig may follow either through the dorsal or ventral thalamus. Fibers coursing through the ventral thalamic region provide a strong projection to the zona incerta, where a particularly dense terminal field was found in its ventral part (Fig. 8A). A contingent of fibers following this path takes a dorsomedial course, provides a moderate terminal field to Forel's field, and projects to the ventral medial thalamic nucleus, which contained a dense terminal field covering almost its entire rostrocaudal extent (Fig. 8B). Additionally, a small number of projecting axons following this path continues further rostrally to project sparsely to the rostral reticular thalamic nucleus and globus pallidus.

A substantial number of ascending fibers from the lateral SCig projects to and through the dorsal thalamus. As shown in Fig. 8A, at caudal thalamic levels, the lateral

SCig provides a dense terminal field to the lateral part of the parafascicular nucleus and the posterior complex of the thalamus. Proceeding rostrally, fibers following this path project to other intralaminar nuclei, in particular the central medial and central lateral nuclei, and to a lesser degree, the paracentral nucleus (Fig. 8B). Contiguous with the projection to these intralaminar nuclei, a careful analysis of our material revealed a dense terminal field in the adjacent ventral region of the lateral part of the mediodorsal nucleus (Fig. 8B). Moreover, in the dorsal thalamus, the lateral SCig also provides a clear terminal field to the ventral anterior-lateral complex of the thalamus (Fig. 8B), in addition to relatively sparse projections to a number of other nuclei, including the lateral dorsal nucleus, nucleus reuniens and rhomboid nucleus (Fig. 8B).

Projections of the medial part of the SCig. We have further examined the projections of the medial part of the SCig to compare with the projection pattern just described for the lateral SCig. In two experiments, the PHA-L injections labeled neurons mostly confined to the medial SCig. Both experiments presented a very similar projection pattern, and we chose experiment PHA-LSC#7 to illustrate our results (Fig. 6B). In the SC, we have first observed that fibers arising from the medial SCig provide a dense terminal field in adjacent parts of the deep gray layer (Fig. 9A). From the injection site, projecting fibers follow either a

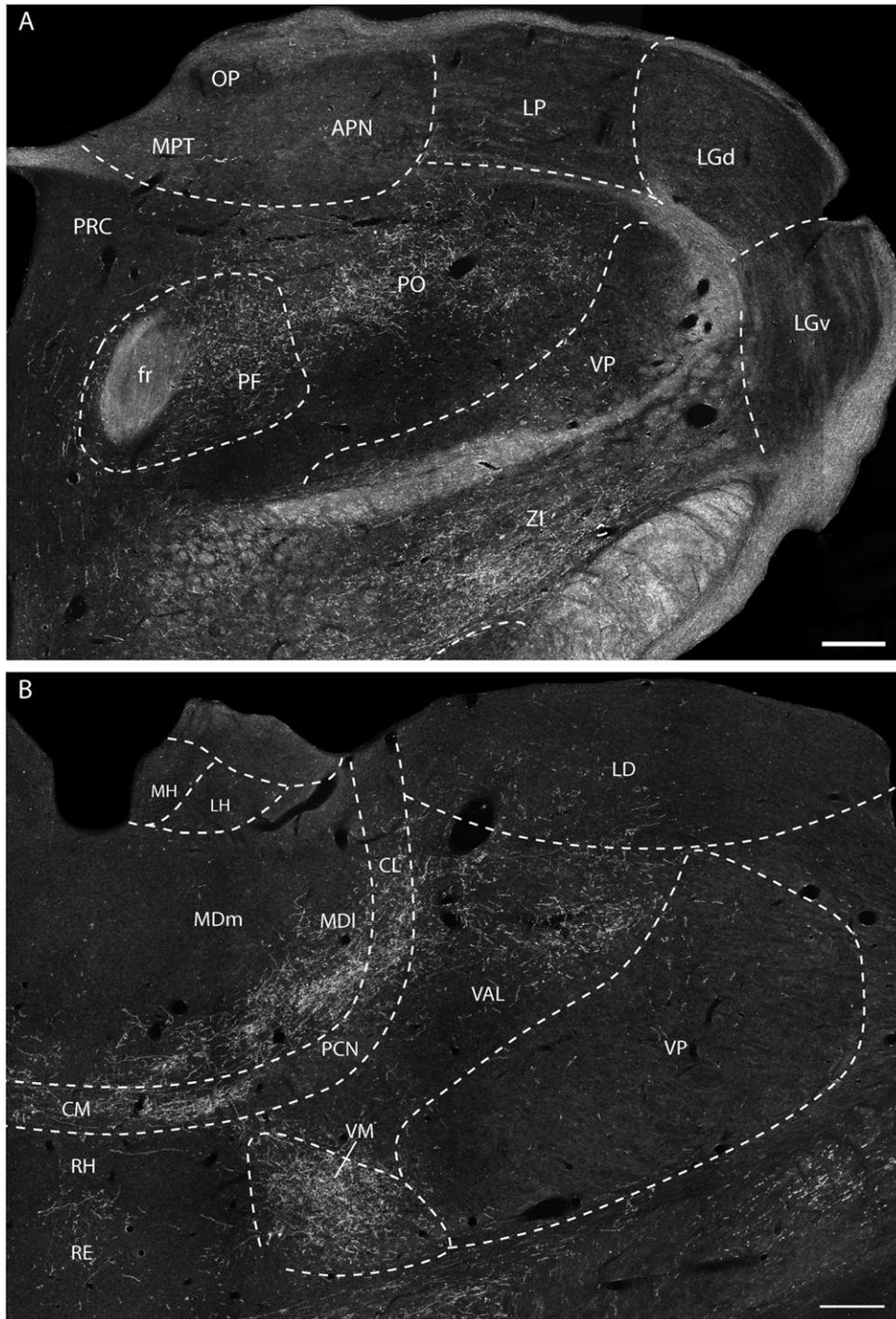


Fig. 8. Experiment 3—Darkfield photomicrographs showing the distribution of PHA-L-labeled axons in experiment PHA-LSC#4, with a PHA-L injection centered in the lateral part of the intermediate gray layer of the SC. (A) Zona incerta and caudal levels of the thalamic region. (B): Intermediate rostro-caudal levels of the thalamic region. Abbreviations: see list. Scale bars=300 μ m.

descending route to the brainstem or an ascending path to the pretectum and diencephalon. At midbrain levels, descending fibers from the medial SCig project densely to the periaqueductal gray (PAG), providing a projection pattern which is very distinct from the one just described for the lateral SCig. Thus, in the rostral PAG, at the levels of the

oculomotor and trochlear nuclei, the medial SCig projects densely to the dorsomedial and dorsolateral PAG columns (Fig. 9A), and proceeding caudally, descending medial SCig fibers provide a significant projection to the ventrolateral PAG column (Fig. 9C). In full agreement with previous studies (Redgrave et al., 1987), the medial SCig

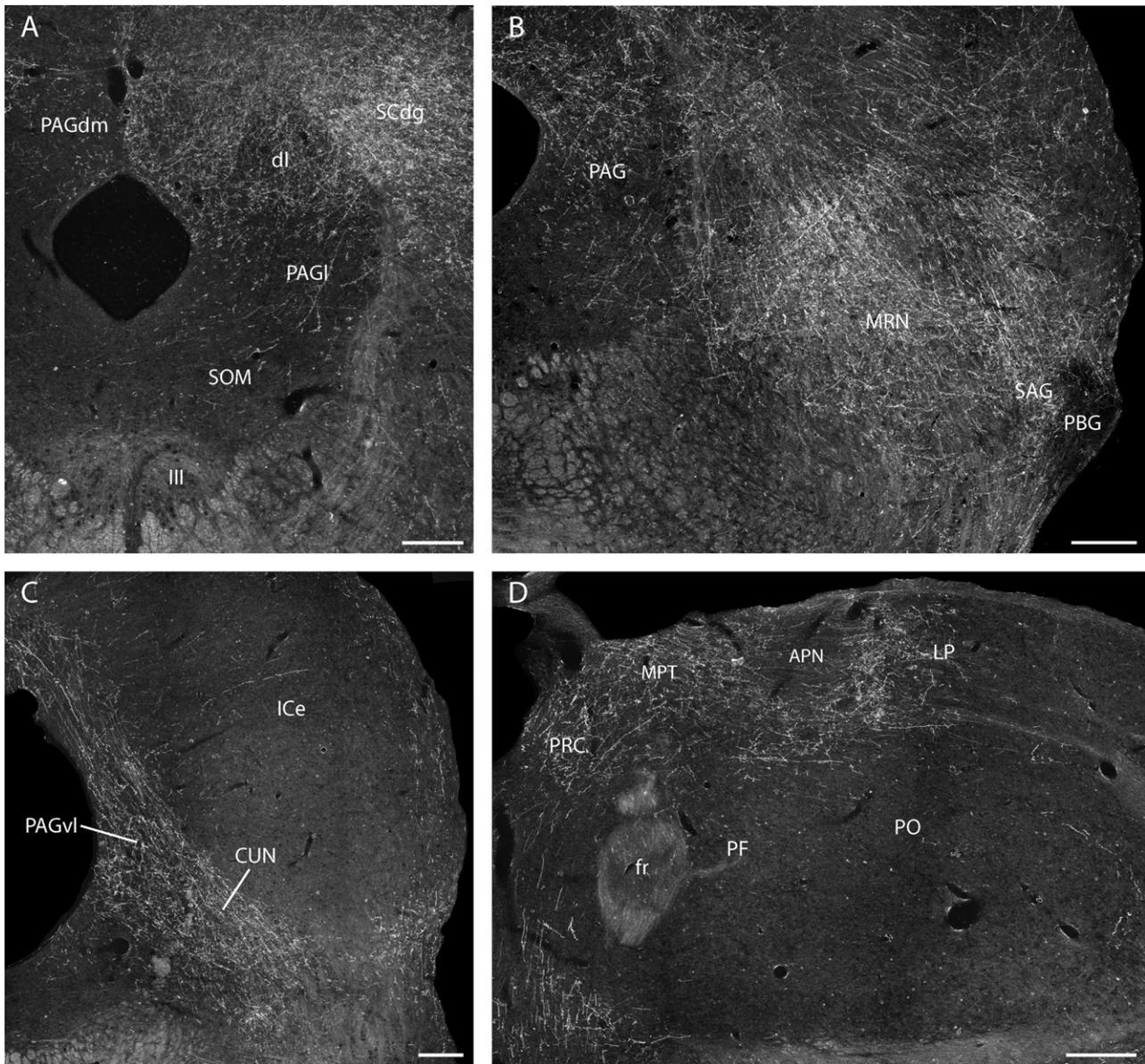


Fig. 9. Experiment 3—Darkfield photomicrographs showing the distribution of PHA-L-labeled axons in experiment PHA-LSC#7, with a PHA-L injection centered in the medial part of the intermediate gray layer of the SC. (A) Rostral levels of the periaqueductal gray. (B) Caudal levels of the mesencephalic reticular nucleus. (C) Caudal levels of the periaqueductal gray and cuneiform nucleus. (D) Precommissural nucleus, pretectal region, and caudal levels of the thalamic region. Abbreviations: see list. Scale bars=300 μm .

provides a robust ipsilateral path to a number of mesencephalic and pontine regions, and in contrast to the lateral SCig, does not seem to contribute significantly to the descending crossed path following the predorsal bundle. At caudal mesencephalic levels, fibers following the uncrossed descending path provide a dense projection to a midbrain region that corresponds to the lateral mesencephalic reticular formation (Fig. 9B). Moreover, at these levels, a contingent of fibers course ventrolaterally to provide a dense projection to the region of the nucleus sagulum, adjacent to the medial border of the parabigeminal nucleus, which, curiously, tends to be avoided by the anterogradely labeled axons (Fig. 9B). Caudal to these lev-

els, ipsilateral descending fibers provide a dense terminal field to the cuneiform nucleus (Fig. 9C), and a contingent of these fibers takes a dorsal course providing a plexus of labeled axons and terminals to the shell region surrounding the external nucleus of the inferior colliculus (Fig. 9C). Descending fibers following this path project to the pontine region and form a distinct terminal field close to the lateral border of the rostral part of the pontine reticular nucleus. Proceeding caudally, part of these fibers continue to provide a relatively sparse projection to ventral parts of the caudal pontine reticular nucleus, which seems to be the caudalmost brainstem site labeled in our medial SCig PHA-L injections.

Compared to what we have described for the lateral SCig, ascending projections from the medial SCig have a more limited set of projections, providing a quite distinct projection pattern to the pretectum, thalamus and ventral thalamus. Ascending fibers from the medial SCig may course either through the ventral or the dorsal thalamus. Fibers projecting through the ventral thalamus provide a substantial projection to the region surrounding the lateral border of the cerebral peduncle, encompassing the parapeduncular nucleus and the lateral end of the subparafascicular nucleus, in addition to a clear terminal field to the medial zone of the ventral lateral geniculate complex. A small number of fibers following this path may continue rostrally to project sparsely to the rostral zona incerta and nucleus reuniens. Ascending fibers coursing through the dorsal thalamic path initially provide a clear projection to the precommissural nucleus and adjacent parts of the medial pretectal nucleus (Fig. 9D). A contingent of these fibers continues through the dorsal thalamus and provides conspicuous terminal fields in the medial part of the lateral posterior nucleus (Fig. 9D), and in the suprageniculate nucleus of the medial geniculate complex. Moreover, fibers ascending through this path may also project sparsely to a number of dorsal thalamic targets, including the paraventricular thalamic nucleus, the lateral dorsal nucleus, the central medial and central lateral intralaminar nuclei, and the reticular nucleus.

DISCUSSION

The results of behavioral, neuronal immediate early gene activation and lesion experiments showed that the lateral part of the SC is particularly critical for the hunting performance. In addition, the present PHA-L experiments present an interesting perspective on how the diverse SC projections may influence hunting behavior, and provide further evidence to characterize the distinct SC functional domains.

First, we have shown that insect hunting is associated with a characteristic increase in Fos labeled cells in the superficial regions of the lateral SCig (i.e., layer a and superficial layer b), in addition to a moderate, but significant, Fos increase in the medial part of SCig. Fos-upregulation in the medial SCig may also be observed in other behavioral activities, including maternal behavior and foraging (Mota-Ortiz and Canteras, unpublished observations); however, to our knowledge, only predatory hunting seems to present such a characteristic Fos increase in the lateral SCig.

The SC is classically known as a locus where signals from different senses are combined and used to guide adaptive motor responses (Grobstein, 1988; Dean et al., 1989; King, 2004; Boehnke and Munoz, 2008; Felsen and Mainen, 2008). The superficial layer of the SC is exclusively visual, whereas the deep layers contain neurons that are responsive to visual, auditory and/or somatosensory stimuli (Saito and Isa, 2007). The neurons in the superficial layer of the SC are organized into a retinotopically coded map of the contralateral visual space and may project to

the intermediate and deep layers (Saito and Isa, 2007). In the intermediate and deep collicular layers, visual inputs merge with concurrent inputs from the extrastriate cortex, in addition to signals from auditory and somatosensory systems, and with information about motor plan from the basal ganglia (Boehnke and Munoz, 2008).

In particular, neurons in the lateral SCig respond chiefly to contralateral vibrissal stimulation and small dark moving objects in the lower rostral and lateral visual field (Dean et al., 1989). In the context of predatory hunting, it is noteworthy that the ventrolateral striatum, which is particularly activated during predatory hunting (Comoli et al., 2005) and likely to implement the motor pattern seen during prey capture and handling (dos Santos et al., 2007), may also influence the lateral SCig through its dense projections to the lateral part of the substantia nigra pars reticulata (Groenewegen et al., 1993). In fact, it has been shown that the responses to vibrissal stimulation and small moving objects may be enhanced by intrastriatal injection of glutamate, which silences the GABAergic cells in the pars reticulata of the substantia nigra projecting to the superior colliculus, thus releasing the collicular neurons from the tonic inhibitory nigral influence (Chevalier et al., 1985). Therefore, the lateral SCig is in a position to integrate critical sensory information to prey detection, along with the basal ganglia inputs, likely to convey the motor plan related to the predatory stereotyped sequence of actions. Recent studies have also shown that the SC may be critical for orienting, triggered by olfactory stimuli (Felsen and Mainen, 2008), a particularly critical fact for predatory hunting, since prey olfactory cues are likely to have an important motivational role to trigger the behavioral responses (Comoli et al., 2005).

Bilateral lesions of the lateral parts of the SC (SCI) produced multiple deficits during prey hunting. SCI-lesioned rats presented a significant delay in starting to chase the prey and longer periods engaged in other behaviors than predatory hunting. During prey hunting, SCI-lesioned animals showed clear deficits to orient themselves toward the moving prey and lost the stereotyped sequence of actions seen for capturing, holding and killing the prey.

The SC has been considered a critical part of the circuitry for sensory-guided orienting decisions (Grobstein, 1988; Dean et al., 1989; King, 2004; Boehnke and Munoz, 2008; Felsen and Mainen, 2008). As previously discussed, the SCI integrates relevant sensory information for prey detection, which should serve the SC to generate the signal of priority to hunt the prey (Boehnke and Munoz, 2008). Therefore, SCI lesions obviously blunt the resulting signal of priority to hunt the prey, accounting, at least in part, for the delay in starting the prey capture and the increased time spent in other activities. Conversely, both of these deficits may be further related to a general lack of motivation or interest to chase the prey, likely to be mediated by the projections from the SCI to the lateral periaqueductal gray, as we shall discuss more fully below.

It comes as no surprise that SCI-lesioned animals presented a clear deficit to orient themselves toward the mov-

ing prey. On the sensory side, as already mentioned, the lateral SC integrates critical sensory information to prey detection, and on the motor side, a number of studies using either electrical or pharmacological stimulation have shown that the lateral SC may elicit a wide range of contralaterally directed movements involving eyes, ears, whiskers, head and body movements, which may mimic pursuit of a moving object (Dean et al., 1986, 1988; Sahibzada et al., 1986). Indeed, some sites giving pursuit-like movements also give biting or gnawing movements (Kilpatrick et al., 1982). This type of contralateral movements resembling a pursuit of a moving stimulus depends on the crossed descending pathway (Dean et al., 1986; Ellard and Goodale, 1986) that arises particularly from the lateral SC (Chevalier and Deniau, 1984; Redgrave et al., 1986, 1987; Bickford and Hall, 1989; present results).

In addition to the orienting deficits, SCI lesions interfere with the innate ability to capture and handle the prey. The capture procedures seen in SCI-lesioned animals were much less efficient, and the animals tried to seize the prey mostly using the mouth, with little assistance from the forepaws. Moreover, while handling the prey, SCI-lesioned animals 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. The predatory stereotyped sequence of actions seems to be implemented by the ventrolateral striatum (dos Santos et al., 2007). In fact, animals with pharmacological inactivation of the ventrolateral striatum presented a severe deficit in the stereotyped actions seen during prey capture and handling (dos Santos et al., 2007); and, as previously discussed, the ventrolateral striatum may influence the lateral SCig through its dense projections to the lateral part of the substantia nigra pars reticulata (Groenewegen et al., 1993). Therefore, during predatory hunting, the orienting responses integrated in the lateral SCig are likely to be shaped by the basal ganglia motor plan related to the predatory stereotyped sequence of actions. The loss of the stereotyped sequence of actions seen in bilateral SCI-lesioned animals may also suggest the lateral SC as a critical region to convey basal ganglia motor plans related to stereotyped actions of hunting to other brain sites.

Given the important role of the lateral SCig in the control of predatory hunting, we have revisited the projections of this collicular region, and further compared the connection pattern of the medial SCig, which represents a distinct functional domain in the SC.

In full agreement with previous reports (Chevalier and Deniau, 1984; Redgrave et al., 1986, 1987; Bickford and Hall, 1989), the lateral SCig, but not the medial SCig, provides a substantial crossed descending projection that follows the predorsal bundle and projects to a number of pontine and medullary sites, including the paramedian region of the pontine reticular nucleus, the tegmental reticular nucleus, the pedunculo-pontine nucleus, the laterodorsal tegmental nucleus, the periaqueductal region, the gigantocellular and medullary reticular nuclei, the parvocellular reticular nucleus, the facial nucleus, and the medial accessory olive. Although not examined in the present study,

fibers following this path are also known to project to the spinal cord (Redgrave et al., 1986, 1987). As already mentioned, this crossed descending pathway with extensive contacts in the pontine-medullary reticular formation and spinal cord has been considered the tectofugal pathway most likely to relay signals associated with the production of orienting pursuit-like movements (Dean et al., 1986; Ellard and Goodale, 1986).

At midbrain levels, the lateral SCig also projects to a number of midbrain sites, including the lateral column of the periaqueductal gray, the mesencephalic reticular nucleus, and the compact part of substantia nigra. The connections from the deep layers of the SC to the compact part of substantia nigra have been reported in a number of species (Comoli et al., 2003a; McHaffie et al., 2006; May et al., 2009), and are ideally located to relay short-latency unpredicted visual information to midbrain dopaminergic neurons. The SC projection to the midbrain dopaminergic cells is likely to account for their stereotypical short-latency burst of activity, considered as representing reward prediction errors used as teaching signals in motor learning, to promote actions that will maximize future appetitive responses (Redgrave and Gurney, 2006). The SC projection to the lateral column of the periaqueductal gray has also been suggested in a previous retrograde study, and arises from SCig cells in the superficial layer a (Mota-Ortiz et al., 2009). The lateral PAG seems to be a nodal part of a neural circuit involved in the decision-making process between hunting, foraging, and other behavioral responses (Sukikara et al., 2006); therefore, it should represent an important link for the SC to influence the motivation drive to hunt and forage. We have previously shown that NMDA excitotoxic lesions of the lateral PAG, but not other parts of the PAG, produced a dramatic effect in inhibiting insect hunting, an effect thought to be mediated through its projections to the lateral hypothalamic (LHA) region containing melanin-concentrating hormone and orexin neurons (Sukikara et al., 2006). Accordingly, insect hunting upregulates Fos expression in this LHA region (Comoli et al., 2005), which seems to correspond, at least in part, to the LHA region previously described in the pioneering studies of Flynn and colleagues (Wasman and Flynn, 1962) and Panksepp (1971) to elicit predatory-like quiet biting attacks in cats and rats. Strengthening the link between prey target detection and the LHA region related to predatory attack, Bandler and Flynn (1971) had shown that predatory attack elicited by electrical stimulation of the hypothalamus lunges more frequently toward a mouse presented to the contralateral visual eye field. Interesting enough, the effects of this LHA region on predatory attack is likely to be mediated through its descending projections to the ventral tegmental area (Bandler et al., 1972; Proshansky et al., 1974).

The lateral SCig provides an extensive ascending projection to the thalamus and zona incerta. The projection to the zona incerta has been previously documented in earlier studies (Chevalier and Deniau, 1984; Shammah-Lagnado et al., 1985). The present results are also in general agreement with previous studies that documented

the SC projections to the thalamus (Chevalier and Deniau, 1984; Yamasaki et al., 1986; Bickford and Hall, 1989; Krout et al., 2001), but revealed important differences regarding some of the main SC thalamic targets. As documented in previous studies (Yamasaki et al., 1986; Krout et al., 2001), the present data show that the lateral SCig provides dense projections to intralaminar nuclei, where conspicuous terminal fields were found in the lateral part of the parafascicular nucleus and in the central medial and central lateral nuclei. However, in contrast to these earlier reports, a careful analysis of our PHA-L material revealed that the intralaminar paracentral nucleus tended to be avoided by the anterograde labeled fibers, which, instead, provided a dense terminal field in the contiguous ventral region of the lateral part of the mediodorsal nucleus, a projection also reported in earlier studies (Groenewegen, 1988). In agreement with previous observations, we have also found that the lateral SCig provides massive inputs to the ventral medial thalamic nucleus and also projects densely to the posterior complex of the thalamus (Herkenham, 1979; Yamasaki et al., 1986; Krout et al., 2001). Moreover, our PHA-L analysis also disclosed an important projection from the lateral SCig to the ventral anterior-lateral complex of the thalamus.

Taken together, these results suggest that the lateral SCig should have a strong influence in modulating activity in the basal ganglia and cerebral cortex through its projections to the thalamic nuclei. Hence, through the connections to the lateral parafascicular nucleus, the lateral SCig is in a position to modulate the ventrolateral striatum (Van der Werf et al., 2002), and therefore influence the striatal region involved in implementing the stereotyped actions seen during prey capture and handling (dos Santos et al., 2007). The connection from the lateral SCig to the parafascicular nucleus closes a basal ganglia loop formed by the ventrolateral striatum, the lateral part of the substantia nigra pars reticulata, the lateral SCig and the parafascicular nucleus. These closed-loop connections are a fundamental feature of architecture linking the basal ganglia not only with cortical domains but also with subcortical systems, and are said to provide an elegant solution to the “selection problem” by prioritizing simultaneous, potentially incompatible inputs (McHaffie et al., 2005). In the present case, this basal ganglia loop should help to adequate the hunting motor pattern to the constant change in velocity and direction of the moving prey.

Through the connections to the lateral parafascicular nucleus, the ventral medial thalamic nucleus, the ventral anterior-lateral complex of the thalamus, and the posterior complex of the thalamus, the lateral SCig may also have access to the motor and somatosensory cortex (Herkenham, 1979; Price, 1995; Van der Werf et al., 2002). In this regard, it is noteworthy that, at least in part, these projections may arise from collaterals of the descending crossed path (Chevalier and Deniau, 1984; Bickford and Hall, 1989), which are likely to supply the efferent copy of the motor command that the SC sends to premotor sites in the brainstem and spinal cord. In addition, through the connections to the central medial and central lateral intralami-

nar nuclei, and the lateral part of the mediodorsal thalamic nucleus, the lateral SCig has access to prefrontal cortical regions, including the supplementary motor, anterior cingulate, and prefrontal cortices (Groenewegen, 1988; Price, 1995; Van der Werf et al., 2002), which, at the top of the perception-action cycle, are thought to play a critical role in behavioral planning (Heidbreder and Groenewegen, 2003). In line with this view, several pieces of data suggest that the SC is important not only for executing movements but for planning them as well (Carello and Krauzilis, 2004). Finally, the main thalamic target of the lateral SCig appears to be the ventral medial thalamic nucleus, which, apart from a relatively small projection to layers III and V of the primary motor cortex, provides a dense projection to the most superficial part of layer I extending over almost the entire neocortex (Herkenham, 1979), influencing, perhaps, the general levels of arousal.

As summarized in Fig. 10, the present data show that the lateral SCig should play a critical role in predatory hunting, and besides providing the well-documented descending crossed pathway to premotor sites in brainstem and spinal cord, projects to a number of sites potentially influencing important functions to the context of predatory behavior, such as general levels of arousal, motivational level to hunt or forage, behavioral planning, motor learning to maximize future appetitive responses, appropriate selection of the basal ganglia motor plan to hunt, and motor output of the primary motor cortex.

In contrast to what we have found for the lateral SC lesions, medial SC lesions produced a small deficit in predatory hunting, and the only insect-hunting parameter recorded that differed significantly from the sham-lesioned animals was an increased delay to start hunting. The reason for this increased delay to start chasing the roaches is not clear at the moment, and perhaps, may be related to the fact that medial SC lesions would interfere with general levels of arousal (Redgrave and Dean, 1985; Dean et al., 1991). The medial SC is functionally distinct from the lateral SC, and is capable of producing a fully integrated defensive response when appropriately triggered. The medial SC contains cells that respond to visual stimuli that rapidly expand in size in the upper part of the visual field, such as a looming shadow signaling potential collision or a potential predator approach (Dean et al., 1989). Differently from what had been discussed for the lateral SC, stimulation of the medial SC produces defense-like responses, including sustained freezing or flight, increased blood pressure and heart rate, analgesia, and EEG cortical arousal (Redgrave and Dean, 1985; Dean et al., 1989). These responses are thought to be mediated by the uncrossed descending projections to the periaqueductal gray, the cuneiform nucleus, and mesencephalic and pontine reticular nuclei (Mitchell et al., 1988). In our cases with PHA-L deposits centered in the medial SCig, we were able to confirm the previous findings from earlier studies on the SC descending uncrossed pathway projection pattern (Redgrave et al., 1987), and further clarified its projection fields to the PAG. Interestingly, the medial SCig projects densely to the PAG regions chiefly responsive to exposure

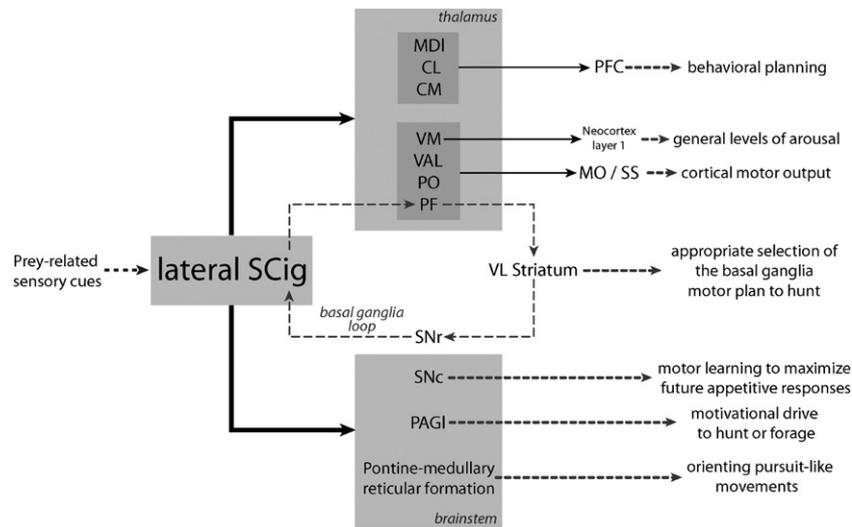


Fig. 10. Summary diagram illustrating the major axonal outputs of the lateral part of the intermediate gray layer of the SC (lateral SCig), arranged to emphasize their possible functional significance, as discussed in the text. Abbreviations: see list.

to a live predator (Canteras and Goto, 1999; Cezário et al., 2008). These PAG regions have been thought to be critically involved in organizing the entire range of anti-predatory defensive responses (Cezário et al., 2008). In addition, compared to what we had seen for the lateral SCig, our PHA-L investigation showed that the medial SCig has a more limited set of projections to the thalamus, and projects very sparsely to thalamic sites related to the control of motor planning or motor output, but instead appears to provide conspicuous inputs to the lateral posterior nucleus and the supragenulate nucleus of the medial geniculate complex. In the context of anti-predatory defense, it is noteworthy that the supragenulate nucleus would be in a position to convey predatory visual threats from the medial SC to the lateral nucleus of the amygdala (Price, 1995), which is an amygdalar site responsive to predator presence and seemingly integrates a range of predator-derived sensory cues processed in the visual and auditory association cortical areas (Canteras, 2002).

CONCLUSION

The results of behavioral, neuronal immediate early gene activation, lesion, and neuroanatomical experiments indicate that the lateral SC is critical for predatory hunting. Lateral SC lesions produced severe deficits in the hunting performance, and the anatomical data suggest that the lateral SC is in a central crossroad of the circuits organizing predatory hunting. On one side, the lateral SC has access to the premotor sites involved in controlling orienting pursuit-like movements, and on the other side, it is in a position to influence a wide range of functions potentially critical for hunting behavior, including arousal, motivation, motor learning, behavioral planning, and cortical motor output. Further studies are obviously necessary to understand how the lateral SC controls each one of these functions. Additionally, the present anatomical observations give further support to characterize the medial and lateral

SC as distinct functional domains in the SC, and to understand how they could mediate the decision to pursue or escape from a given stimulus.

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