General Discussion and Summary

Introduction
The present thesis presents new direct spinal efferent pathways from and afferent pathways to the periaqueductal gray (PAG). The PAG plays an important role in the control of emotional behavior, such as defense behavior (fight, freezing and flight), micturition and sexual behavior. The PAG is also well known for its role in the control of nociception: stimulation of the PAG can inhibit pain. The present work has been done with the aim to precisely determine the pathways that the PAG uses to control such emotional behaviors.

The methods necessary to reveal new pathways are the anterograde and retrograde tracing methods. These methods are based on injecting small amounts of tracer into an intact and alive central nervous system. Since, after a certain survival time the central nervous system has to be perfused and cut into sections to visualize the tracer, such studies according to ethical standards cannot be performed on humans. Therefore, all studies presented in this thesis are performed in cat. Concerning the emotional motor system, the results in cat are assumed to be of great relevance for the understanding of this system in humans. The central nervous system of the cat, and especially its brainstem part, is very similar to that of the human. Recent work from our department (Blok et al., 1997a,b, Blok et al., 1998) revealed, using PET scanning techniques in humans, that the brainstem structures involved in the regulation of micturition are found in similar locations in cat and human.

In the last few decades the efferent and afferent connections of the PAG have been studied by many research groups. Several of these studies, however, were ‘purely anatomical’ as they presented pathways to and from the PAG, without considering a functional perspective. Others were studying the efferent and afferent pathways of the PAG exclusively in the context of the control of pain. All these studies produced relevant information, but lacked the precise data to result in a thorough understanding of the PAG with all its different functions. It appeared that for such an understanding of the efferent and afferent pathways it was necessary to study various parts of the PAG combined with all separate C1-Coc2 spinal segments. All other studies involved only a selected part of the PAG and/or a few segments of the spinal cord.

The results of the present work not only resulted in a much more detailed description of the direct spinal efferent and afferent pathway of the PAG, but also in a different view on the functions of the PAG in the context of survival and sexual behavior.

Direct spinal efferents of the PAG
The general idea was that there exist only limited projections from the PAG directly to the spinal cord. Earlier studies (Holstege, 1988a,b) showed the existence of a direct efferent projection from the PAG to the intermediolateral cell column of T1 and T2, possibly involved in pupil dilatation during emotional behavior (Fig. 1). The present thesis gives a precise description of another PAG spinal pathway that originates in the lateral and ventrolateral PAG (Chapter 1) and, furthermore, it shows that there exists a new, third, PAG-spinal pathway that originates from cells at the dorsal border of the PAG (Chapter 2).

PAG projection to lamina VIII and the medial part of lamina VII
In chapter 1, using the retrograde and anterograde WGA-HRP tracing techniques, it is concluded that this PAG-spinal pathway has its origin in the ventrolateral and lateral PAG and...
in the laterally adjacent mesencephalic tegmentum. The relatively large neurons giving rise to this projection are located in a rostrocaudally oriented column within this area, with the highest density of neurons located just rostral to the trochlear nucleus. The fibers terminate, predominantly ipsilaterally, in lamina VIII and the medial part of lamina VII of the cervical cord. However, terminations were also found in the same laminae in the thoracic, lumbar, and, scarcely, in the sacral cord segments. As lamina VIII and the medial part of lamina VII are known to contain premotor interneurons of axial muscles of neck and trunk, and of proximal muscles of arm and leg (Rustioni et al., 1971; Sterling and Kuypers, 1968; Molenaar et al., 1974; Molenaar, 1987), this particular PAG-spinal pathway must be involved in the control of axial and proximal musculature. Comparing these anatomical results with the results of physiological studies in which the lateral and ventrolateral PAG of the cat was stimulated (Bandler and Carrive, 1988; Zhang et al., 1990; Bandler et al., 1991) it is hypothesized that this pathway plays a role in threat display, as part of defense behavior.

**PAG projection to the spinal central canal area**

Some cases of the retrograde tracing study presented in chapter 1 showed, besides the neurons in the lateral and ventrolateral PAG, some faintly labeled small neurons at the dorsal border of the periaqueductal gray. These neurons had never been reported before. In the study of chapter 2 the retrograde WGA-HRP tracing technique was used to determine where exactly this group of neurons is located. The anterograde tracing technique was used to reveal to which parts of the spinal cord these dorsal border cells projected. It appeared that this cell group consisted of relatively small, oval or spindle shaped neurons in the caudal dorsomedial and dorsolateral PAG and more rostrally in the midline area. Most of the cells are located exactly at the dorsal border of the dorsolateral PAG, and a few just within or just outside the dorsomedial PAG. However, most peculiar were the results of the anterograde study. The anterograde tracing study showed that the termination sites of these neurons are in the area surrounding the central canal of the C4-T8 spinal segments, and hardly any fibers were found to terminate on neurons in this central canal area. Electron microscopy showed that the terminals were in the neuropil of the subependymal area of the central canal and in the vicinity of the basal membranes of the capillaries located laterally to the central canal. The function of this group of neurons at the dorsal border of the PAG and projecting to the spinal central canal area is unknown. However, the termination in the subependymal area of the central canal suggests that the dorsal border PAG neurons might be involved in the release of transmitter or peptide hormones into the cerebrospinal fluid. The terminations in the vicinity of the basal membranes of the capillaries near the central canal might indicate some kind of neurosecretory function of the PAG. This is an assumption based on the situation in the hypothalamo-hypophysial projection system in which neurosecretion takes place in the neurohypophysis.

It has never been suggested before that the PAG, similar to the hypothalamus, has a neurosecretory function, but the PAG and the hypothalamus are anatomically in the same position, close to cerebrospinal fluid regions and functionally alike as both are integrative systems for basic emotional responses. Perhaps the PAG is also similarly organized in using neurosecretory pathways to elicit such responses.

**Direct spinal afferents to the PAG**

The general idea was that the many direct afferents from all levels of the spinal cord to the PAG are mainly involved in the relay of pain information. Only recently it was suggested that not all direct spinal projections to the PAG are involved in pain, but that those
Fig. 1. Schematic overview of the efferent pathways of the PAG to more rostrally located structures and to more caudally located structures in brainstem and spinal cord. The functions in which each of the descending projections might be involved are also indicated.
from the lumbosacral cord might be involved in the relay of information considering mic-
turition and mating behavior (Blok, 1995; 

The retrograde tracing studies of chapters 3, 
4, 5 and 6 of this thesis present a detailed map 
of the complete spino-PAG projections in the 
cat.

**Total number of spino-PAG neurons**

A remarkable finding in the studies on the 
direct spinal afferents of the PAG was the to-
tal number of neurons involved. Although it 
was not the aim of the study to determine the 
absolute number of spino-PAG projecting 
neurons, the results show that the spino-PAG 
projections consist of at least 15,000 neurons 
in the C1-Coc2 spinal cord (see Chapter 6). 
An earlier study in the monkey estimated this 
number to be 10,000 (Wiberg et al., 1987), 
while in the cat it was estimated to be only 
3,000 (Wiberg and Blomqvist, 1984). The 
results of chapter 3 of this thesis explain the 
large differences between this earlier found 
number in the cat and the present results. In 
this chapter large segmental differences are 
shown in the number of spino-PAG neurons, 
even between adjacent or nearby segments. 
For instance, C1 contains almost twice as 
many spino-PAG neurons as C2, and S2 twice 
as many as S3. Furthermore, it shows that 
certain parts of the PAG receive many more 
spinal afferents than other parts, as injecting 
different parts of the PAG led to different 
numbers of labeled spinal neurons. In the 
present study the estimated total number of 
spino-PAG neurons in the cat (15,000) was 
derived from injections involving major parts 
of the PAG. The earlier estimation (3,000) of 
Wiberg and Blomqvist (1984) was derived 
from injections targetting the intercollicular 
area, while their injections involved only a 
small part of the PAG. Furthermore, in con-
trast to the present estimation, which was 
derived from counting of labeled cells in all 
separate spinal segments, the earlier estima-
tion was based on the counting of labeled cells 
of only 12 spinal segments and the authors 
assumed that the numbers in other segments 
could be interpolated from the counted numbers.

**Comparison of the number of spino-PAG 
cells and number of spino-thalamic cells**

The number of at least 15,000 spino-PAG 
cells is even more remarkable when compar-
ing it with the much better known ascending 
pathway: the spinothalamic tract (STT). A 
quantitative study of the STT in the monkey, 
including all separate spinal segments 
(Apkarian and Hodge, 1989), estimated the 
number of STT cells as more than 18,000. In 
the cat a study including all separate spinal 
segments does not exist, but Craig et al. 
(1989) counted labeled STT cells in the C5-
C7 and L5-S2 segments, which led them to 
the estimation that the C3-Coc spinal cord 
contains about 5,000 STT cells. The present 
study shows that the C3-Coc spinal cord con-
tains at least 10,000 spino-PAG cells. It means 
that in the cat the spino-PAG pathway is 
a major ascending tract and at least twice as 
strong as the spinothalamic tract!

**Five separate spino-PAG systems**

A comparison between the number of cells 
located in lamina I that project to the PAG 
and the number of cells located in other lami-
ae that project to the PAG demonstrates that 
the lamina I-PAG projection provides only a 
minor portion of all spinal projections to the 
PAG (see Chapter 5). Apparently the spino-
PAG projection is not one general ascending 
system of which the only function is the relay 
of nociception. In chapter 6 of this thesis a 
large study is designed to describe all these 
components, and not merely that of lamina I. 
The results of this study show that the spino-
PAG projections consist of at least five ana-
tomically separate systems, probably with 
separate functions (Figs. 2 and 3). System I 
originates in laminae I and V throughout the 
length of the cord, terminates in all parts of 
the PAG, and is probably involved in
System II begins in the ventrolateral part of laminae VI-VII of the upper cervical spinal cord and terminates in the ventrolateral and lateral parts of the rostrocaudal PAG, and in the deep tectum. System III originates in lamina X of the thoracic and upper lumbar cord, and terminates in the PAG as well as in the deep tectum. System IV originates in the medial part of laminae VI-VII of the lumbosacral cord and projects predominantly to the lateral and ventrolateral caudal PAG. It might play a role in conveying tactile stimuli to the PAG during mating behavior. System V begins in the lumbosacral cord and terminates mainly in the central portion of the lateral and ventrolateral caudal PAG, and probably relays information concerning micturition (e.g. bladder filling) and mating behavior.

No somatotopic organization in the spino-PAG projection
Wiberg and Blomqvist (1984) showed that after large injections in the lumbosacral enlargement, more anterogradely labeled fibers terminated in the intermediate and caudal PAG than after large injections in the cervical enlargement. This led them to conclude that the spino-PAG projection system is somatotopically organized. Indeed, the present results in chapter 6 show larger numbers of lumbosacral neurons after injections in the intermediate and caudal PAG than after large injections in the cervical enlargement. However, these findings are not the result of a somatotopic organization of the spino-PAG projections, but of the fact that there are two spino-PAG systems that contain lamina I neurons. A tracer injection in lamina I of C6-C8 involves system I and will result in labeled fibers in the entire rostrocaudal PAG. An injection in lamina I of L6-7, however, involves system I, projecting throughout the rostrocaudal PAG, but also involves system V. System V does not project to the entire rostrocaudal PAG, but exclusively to the caudal/intermediate PAG. Therefore, an injection in L6-7 will lead to denser labeling in the caudal PAG than an injection in C6-8.

Comparison of the five spino-PAG systems with the spinothalamic tract
The studies presented in this thesis show that the spino-PAG projections probably subserve many functions in addition to nociception. With this knowledge it is tempting to find out whether the spinothalamic projection also includes similar different systems. System I: Comparing the presently described lamina I-PAG projection with the lamina I-thalamic projection in the cat (Zhang et al., 1996), shows a similar spinal distribution of both projection systems, with most neurons located in the enlargements. A comparison of the lamina I-PAG projection with the lamina I-thalamic projection, which has always been thought to be the most important pain pathway, even led to the conclusion that in the cat...
General Discussion and Summary

SYSTEM I
includes 50% of all spinal PAG projecting neurons
originates in laminae I and V throughout the length of the spinal cord
terminates mainly contralaterally
in the ventrolateral and lateral parts of the rostrocaudal PAG

Possible function relay in nociceptive, mechanical and visceral information

SYSTEM II
includes 20% of all spinal PAG projecting neurons
originates in the ventrolateral part of laminae VI-VII of C1-C3
terminates bilaterally, stronger ipsilaterally
in the ventrolateral and lateral parts of the rostrocaudal PAG
and in the deep tectal layers

Possible function unknown

SYSTEM III
includes 2% of all spinal PAG projecting neurons
originates in laminae X of the thoracic and upper lumbar cord
terminates mainly contralaterally
in the PAG and adjacent deep tectal layers

Possible function unknown

Fig. 2. Schematic overview of the five separate spino-PAG systems
SYSTEM IV
includes 10% of all spinal PAG projecting neurons
originates in the medial part of laminae VI-VII of L4-Coc2
terminates mainly contralateral
mainly in the ventrolateral and lateral parts of the caudal PAG

Possible function neurons contain estrogen receptors
relay for tactile information that can induce receptive behavior

SYSTEM V
includes 10% of all spinal PAG projecting neurons
originates in the lateral part of lamina I of L6-S2 and in laminae V-VII
and X of S1-S3
terminates mainly contralaterally
in the central part of the ventrolateral and lateral caudal PAG

Possible function relay of information concerning micturition and mating behavior
Fig. 3. Schematic overview of the afferent pathways to the PAG from more rostrally located structures and from more caudally located structures in brainstem and spinal cord.
there are three times as many lamina I neurons projecting to the PAG than to the thalamus (Chapter 4).

System II: The strong upper cervical PAG projection has its counterpart in the spinothalamic tract (rat: Granum, 1986; Kemplay and Webster, 1986; cat: Carstens and Trevino, 1978a; Carstens and Trevino, 1978b; Comans and Snow, 1981; monkey: Apkarian and Hodge, 1989), because the upper cervical cord, not including the lateral cervical nucleus, contains about 40% of all spinothalamic neurons (Kemplay and Webster, 1986). As in system II of the spino-PAG projection, a large portion of these spino-thalamic cells is located ipsilaterally in the lateral laminae VI-VIII; they project bilaterally to the intralaminar nuclei and mainly ipsilaterally to the border area between the ventrobasal complex and nucleus ventralis lateralis (Carstens and Trevino, 1978b, Comans and Snow, 1981). Surprisingly little attention has been paid to this major part of the spinothalamic tract, neither to its precise organization, nor to its specific function.

System III: Although some studies show in their figures the existence of lamina X-thalamic cells (Carstens and Trevino, 1978; Apkarian and Hodge, 1989), no special attention is given to their segmental location, nor to their possible function.

System IV: System IV also has its counterpart in the spinothalamic tract, because neurons in the medial lamina VII of the lumbosacral cord project to the contralateral caudal thalamus (Trevino and Carstens, 1975; Carstens and Trevino, 1978a; Willis et al., 1979). These neurons respond strongly to pressure on the proximal hindlimbs and are characterized by wide-spread inhibitory receptive fields (Menetrey et al., 1984).

System V: None of the studies on the cells of origin of the spino-thalamic tract focussed on the lamina X-thalamic projection.

Epilogue

The mesencephalic periaqueductal gray (PAG) plays an integrative role in emotional responses necessary for basic survival. Functional parts of these responses are the control of pain, micturition and sexual behavior. The discovery of all the neuroanatomical pathways by which the PAG elicits these behaviors would give a much better insight in the clinical problems related to these behaviors. In the field of pain research the spinothalamic tract gets almost all attention, while the role of the direct spino-PAG projections in this field is almost completely obscure. The results presented in this thesis make it very obvious that this neglect is unjustified, since a large part of the emotional effects of ascending nociceptive information might be due to the relay through the PAG.

In the context of micturition urodynamic investigation have shown that the majority of incontinence of humans older than 65 years is caused by lesions in the brain. As recent studies in cat and humans have shown that the PAG plays an important role in micturition, further research in this field seems to be essential to finally understand the causes of incontinence and come to solve this huge world-wide problem.

Relatively little research attention has been given to the problem of sexual disorders, mainly because these problems are often considered to be ‘psychological’ and not medical problems. However, sexual behavior is a typical emotional behavior similar to freezing, flight and flight or micturition, and is under complete control of the emotional motor system. When in the near future the ‘hardware’ component of sexual behavior will be uncovered, this will undoubtedly lead to a different approach of the problems in this field.