Three times as many lamina I-PAG

Chapter 4

Three times as many Lamina I Neurons project to the Periaqueductal Gray than to the Thalamus; a Retrograde Tracing Study in the Cat

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Abstract
The number and distribution of lamina I neurons projecting to the periaqueductal gray (PAG) were examined by a retrograde tracing study in the cat. WGA-HRP injections in the intermediate and caudal PAG resulted in as much as 1600 labeled lamina I neurons throughout the length of the spinal cord, counted in a 1:4 series of sections. The lamina I-PAG projection was predominantly contralateral and most labeled lamina I neurons were found in the enlargements. Comparing these results with the number of lamina I-thalamic neurons leads to the conclusion that in the cat about three times as many lamina I neurons project to the PAG as to the thalamus. Considering this, one can conclude that the spino-PAG system is a virtually neglected area in pain research.

Introduction
Lamina I of the spinal cord plays an essential role in conveying information about pain and temperature. The great majority of lamina I neurons receive Aδ and C fiber input and are nociceptive specific (Willis and Coggeshal, 1991). The spinothalamic tract (STT) is often considered as the main ascending spinal system for pain information. STT neurons are located both in the dorsal and ventral horn of all spinal segments, but, except for the upper cervical cord, about half of the STT neurons are located in lamina I (Craig et al., 1989; Apkarian and Hodge, 1989).

Another area involved in pain regulation and that receives projections from lamina I is the mesencephalic periaqueductal gray (PAG). Earlier retrograde tracing studies in rat (Menetrey et al., 1982; Liu, 1983, Harmann et al, 1988; Lima and Coimbra, 1989; Keay et al., 1997), rabbit (Meller and Dennis, 1986), cat (Keay and Bandler, 1992; Vanderhorst et al., 1996) and monkey (Mantyh, 1982; Wiberg et al., 1987, Zhang et al., 1990; Blomqvist and Craig, 1991) showed the presence of lamina I-PAG cells. However, none of these reports precisely describes the total lamina I-PAG system. Therefore, the present study examined the lamina I-PAG system throughout the cord, with the aim to estimate the total number of lamina I-PAG neurons and to give a description of their segmental distribution.

Materials and Methods
Retrograde tracing study
In four cases (2155, 2159, 2367 and 2385) large injections of approximately 100 nl wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP; 2.5%) were placed in the PAG. In one control case (2338), WGA-HRP was injected in the deep tectal layers laterally to the PAG. The surgical procedures, pre- and postoperative care, handling and housing of the animals were in accordance with protocols approved by the Committee of Animal Experiments of the Faculty of Medicine of the University of Groningen. The animals were initially anesthetized with ketamin (Nimatek, 0.1 ml/kg i.m.) and xylazine (Sedamun, 0.1 ml/kg i.m.), after which
they were kept anesthetized by ventilation with a mixture of O\textsubscript{2}, N\textsubscript{2}O and halothane. During surgery, heart rate and body temperature were monitored. Following a survival time of 3 days the animals were initially anesthetized with the ketamin and xylazine i.m., followed by an overdose of 6% pentobarbital sodium (Nembutal; i.p.). The cats were perfused transcardially with 2 l of heparinized saline at 37\textdegree C, directly followed by 2 l of 0.1M phosphate buffer, containing 4% sucrose, 1% paraformaldehyde and 2% glutaraldehyde. After perfusion, the brain and spinal cord were removed, post-fixed for two hours and stored overnight in 20% sucrose in phosphate buffer at 4\textdegree C. Subsequently, the brainstem and the complete (C1-Co2) spinal cord was cut in 40 \textmu m frozen transverse sections. Every fourth (cases 2367, 2385 and 2338) or fifth (cases 2155 and 2159) section

Fig. 1. Schematic drawings of the location of the injection sites. An indication of what is considered the core of the injection sites is shown as the black area.
was incubated according to the tetramethylbenzidine method, dehydrated and coverslipped. From the area containing the injection site an extra series was processed with diamino-benzidine (DAB).

Quantifications of retrogradely labeled neurons
To obtain an overview of the number and distribution of lamina I-PAG neurons, in each processed transverse section all retrogradely labeled lamina I cells were plotted and

Table 1. Number of labeled lamina I cells, counted in a 1:4 series of sections throughout the length of the spinal cord.

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<tr>
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<th>2159 ipsi contra</th>
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<td>1261</td>
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counted. In order to compare the results of the different cases the numbers of the 1:5 processed cases were corrected into 1:4 cases by multiplying with 1.25. Such a correction is allowed, because in both groups an unbiased sample was taken and double counted cells could not occur. The corrected numbers were used in all further analysis. It must be emphasized that the labeled neurons in the lateral cervical nucleus and in the dorsal column nuclei were not included.

Results
A schematic representation of the injection sites is given in Fig. 1. In cases 2159 and 2367 the injection included the entire rostral PAG and in cases 2155 and 2385 both the intermediate and caudal PAG. In case 2338 the injection site was located in the deep layers of the superior colliculus at the level of the rostral and intermediate PAG. The injection extended to only a very limited extent into the lateral part of the lateral PAG.

As many as approximately 1600 and 1200 labeled lamina I neurons were found throughout the cord in cases 2155 and 2385, with injections in the caudal and intermediate PAG (Table 1). A much smaller number (230) of labeled lamina I neurons were found in cases 2367 and 2159, with injections in the rostral PAG, while after an injection in the deep tectal layers (case 2338) only 54 labeled lamina I neurons were present (Table 1).

All lamina I-PAG projecting neurons were located in the dorsal and dorsolateral parts of lamina I, and not in its medial or ventrolateral parts. Only in the sacral segments were lamina I neurons also found more laterally. The lamina I-PAG projection was predominantly contralateral, only 3 to 18% of the labeled lamina I neurons were found ipsilaterally. In all four PAG injected cases most of the labeled lamina I neurons were located in the enlargements (Table 1, Fig. 2). In cases 2155 and 2385, with an injection in the intermediate and caudal PAG, an averaged total of 260 labeled lamina I neurons was found contralaterally in the C5-C8 and 295 in the L5-S1 spinal cord. In the T5-L2 cord less than 20 labeled lamina I cells were found per seg-

![Fig. 2. Graphic showing the segmental distribution of the retrogradely labeled lamina I neurons, after WAG-HRP injections in the periaqueductal gray (cases 2159, 2367, 2155 and 2385) and in the deep tectum (case 2338) in the cat. Labeled neurons were counted in every fourth section. Note that the labeled neurons in the lateral cervical nucleus and in the dorsal column nuclei are not included.](image-url)
Three times as many lamina I-PAG

Discussion

Zhang et al. (1996), in a retrograde tracing study in the cat, precisely described the segmental distribution of the contralaterally located lamina I-thalamic neurons in the C3-Coc4 spinal cord. In three cases they injected cholera toxin subunit B (CTb), and in one case the fluorescent tracer fast blue, unilaterally in the thalamus. The injection sites involved all thalamic areas known to receive lamina I projections, i.e., the nucleus submedius, the ventral aspect of the basal part of the ventral medial nucleus and the borders of the ventral basal complex. In their CTb injected cases serial 50 μm frozen horizontal sections showed an averaged total of 1360 lamina I-thalamic cells in the contralateral C3-Coc4 spinal cord (Zhang et al.; 1996). Most CTb labeled lamina I neurons were found in the enlargements, with an averaged total of 575 cells in the C5-C8 and 301 in the L5-S1 cord. Only five to ten labeled cells were found in each of the T5-L2 segments. The numbers of labeled lamina I neurons in the FB injected case did not differ from the CTb injected cases. It should be noted that in this study (Zhang et al. 1996) the numbers are from series of 1:1 sections, while no corrections for double counted cells were made, because it

Fig. 3. Graphic showing the differences in number and segmental distribution between the lamina I-PAG neurons and lamina I-thalamic neurons in the cat. The numbers of lamina I-PAG neurons are the averaged numbers of cases 2385 and 2155, multiplied by four. The numbers of lamina I-thalamic neurons are derived from Zhang et al., 1996.
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was not the main concern of the study to come
to precise absolute numbers of cells. Further-
more, Zhang et al. (1996) presented their seg-
mental data neglecting the existence of a T13
segment and including a S4 segment, which
does not exist in the cat. We corrected for this
mistake assuming that their data from the L1-
S4 segments in fact belonged to the T13-S3
segments.

A comparison of the number of lamina I-PAG
neurons and the number of lamina I-thalamic
neurons leads to about 1360 contralaterally
projecting lamina I-thalamic cells in the C3-
Coc cord in serial sections without correct-
ing for double labeled cells, and 1080 lamina
I-PAG cells in the same part of the cord in a
1:4 series of sections. Without corrections for
double labeled cells this equals a total of about
4300 contralaterally projecting lamina I-PAG
neurons. This leads to the striking conclusion
that there exist about three times as many
lamina I-PAG neurons than lamina I-thalamic
neurons.

The segmental distribution of the lamina I-
PAG system resembles that of the lamina I-
thalamic system, in that both systems the ma-
jority of cells are located in the enlargements
(Fig. 3). However, in the lamina I-thalamic
system almost twice as many cells are present
in the cervical enlargement than in the lumb-
osacral enlargement. The same is true for
the lamina I-PAG system, but only for the
rostral PAG. In respect to the intermediate and
caudal PAG, the enlargements contain about
equal numbers of lamina I-PAG neurons.

Comparing the results of the present study
with that of Zhang et al. (1996), it was as-
sumed that CTb and WGA-HRP are equally
efficient in labeling lamina I neurons. This
assumption is based on the good agreement
between the two precise retrograde tracing
studies on the lamina I-thalamic neurons in
the monkey. Large thalamic injections with
WGA-HRP (Apkarian and Hodge, 1989) re-
sulted in an estimated total of about 3700
contralaterally projecting lamina I-thalamic
neurons in the C2-Coc cord, while the CTb
study (Zhang and Craig, 1997) showed a to-
tal of about 3600 such neurons in the same
part of the cord. In both studies about 1100
of these lamina I neurons were located in the
cervical enlargement and about 600 in the
lumbosacral enlargement.

Conclusion

The present retrograde tracing study in the
cat showed the existence of many lamina I
neurons projecting to the PAG, far more than
to the thalamus. Apparently, the PAG plays a
very important role in pain regulation. Given
the enormous concentration of research and
teaching on the spino-thalamic system, the
spino-PAG system, which contains a much
stronger lamina I projection, is an undeserved
neglected area in pain research.