Projections from the brain to the spinal cord in the mouse

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Publication Date:
2011

DOI:
https://doi.org/10.26190/unsworks/15290

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Projections from the brain to the spinal cord in the mouse

Huazheng Liang

Supervisor: Prof George Paxinos
Co-supervisor: Prof Charles Watson

This thesis has been submitted in fulfillment of the requirements for the degree of Doctorate of Philosophy (PhD).

School of Medical Sciences
Faculty of Medicine
The University of New South Wales
2011
ACKNOWLEDGEMENTS

I am extremely grateful to my Australian supervisor Scientia Professor George Paxinos for his constant outstanding instruction, generous support and patience for the past three years. His comprehensive knowledge of neuroanatomy has contributed enormously to this thesis and my education. I will never stop learning from him. I also greatly appreciate my co-supervisor Prof Charles Watson for his tremendous, constructive suggestions and kindness. From the start of my PhD, he has talked over the topic with Prof Paxinos and me for so many times and helped me to learn the neuroanatomy, guiding me to get on the right track in the past three years.

During the PhD training in the past 3 years, I conducted all the experiments. My supervisors assisted me in data analysis and writing three publications, specifically revising my manuscripts. Prof George Paxinos also helped to revise my thesis.

Gratitude is also extended to Prof Gulgun Kayalioglu, Associate Prof Pascal Carrive, Dr. Yuhong Fu, Yue Qi, Henry Li, Deyi Duan, Erika Gyengesi, Emma Schofield, Zoltan Rusznak, Timea Bacskai, Mr Peter Zhao and Reuben Png, Dr Laksmi Govindasamy and Ms Robyn Hutchinson for their advice and constant technical support.

To the panel members at UNSW for their wonderful advices.

To Prof Shaoshi Wang, Glenda Halliday, and Dr Yue Huang for introducing me to this wonderful country.

To those who supported me and made my PhD possible, maybe some of them are not at our institute. Far away from home, I felt you are not only keen scientists but also brothers and sisters from all over the world. The experience I had with you is unforgettable. I wish all of you the best for your scientific and personal endeavors.

To my loving and supportive family: my wife, who has sacrificed a lot for my PhD, taking the responsibility of looking after our son by herself; my parents, my brothers and sisters. Despite thousands of miles away, you were and will always be my source of inspiration.
CARE and USE of ANIMALS

The care and use of animals in the research presented in this thesis comply with the Rules Governing the Use of Animals in Research and Teaching at the University of New South Wales with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes Act (1985 and its subsequent amendments). These experiments were approved by the Animal Care and Ethics Committee at the University of New South Wales.
ABSTRACT

Spinal cord projections have been well studied in a variety of mammals, but have not been systematically investigated in the mouse. The present thesis mapped the neurons of the brain that projected to the mouse spinal cord using retrograde and anterograde tracer injections in the spinal cord. Most of the brain regions labeled retrogradely were those reported already for other species (for example: the motor cortex, hypothalamus, red nucleus, vestibular and trigeminal nuclei, and the reticular nuclei). In addition, two nuclei not previously known to project to the spinal cord were identified to project to the spinal cord: the precuneiform and the epirubrospinal nuclei. Another unexpected finding was that unlike reports on the rat, the mouse amygdala projected to the spinal cord as it does in the cat and monkey.

The axonal terminal of the precuneiform nucleus in the spinal cord was investigated through injections of the retrograde tracer to different segments of the spinal cord. Termination of the precuneiform axons was confined to the cervical and upper thoracic segments. The opposite experiment, anterograde tracer injections to the precuneiform nucleus, revealed axonal terminals from this nucleus in the gray matter of the spinal cord. They mainly terminated in lamina 7, to a lesser extent in the laminae 8, 9, and 10 on the ipsilateral side. The density of these terminals tapered in more caudal segments.

The rubrospinal projections were investigated through retrograde and anterograde tracer injections, immunofluorescence, and in situ hybridization. Consistent with studies in other species, labeled neurons were found in both the rostral (diencephalic part) and the caudal (midbrain part) parts of the contralateral red nucleus after cervical and lumbar cord injections of fluoro-gold. Rubrospinal neurons were topographically organized with lumbar-cord projecting neurons more ventrolaterally located. Cell counting showed that about two thirds of the total neurons projected to the cervical cord and about one quarter to the lumbar cord (there are potential overlaps) (total number is 3200.96±230.80). Immunofluorescence staining with SMI-32 antibody showed that approximately 60% of SMI-32 positive neurons were cervical-cord projecting neurons, and 24% are lumbar-cord projecting neurons. SMI-32 positive neurons are mainly located in the medial portion of the caudal part of the red nucleus.
Comparison of the distribution of rubrospinal neurons in the present thesis with in situ hybridization against vGluT2 mRNA (from Allen Brain Atlas website – http://mouse.brain-map.org) showed that the number and location of glutamatergic neurons matched those of rubrospinal neurons. In situ hybridization against the complement component 1, q subcomponent-like 2 gene (C1QL2) (from Allen Brain Atlas website – http://mouse.brain-map.org) shows that many C1QL2 positive neurons might be rubrospinal neurons, especially in the caudal part of the red nucleus. The opposite experiment, anterograde tracer injections to the red nucleus, produced similar findings to those of previous studies in other species. Densely labeled axons were found in lamina 5 and 6/7, whereas sparsely labeled fibers were found in lamina 8. Labeled fibers were also found in the lateral part of lamina 9 where the extensor muscle motor neurons were located in C8-T1 and L5-6. In addition, the ipsilateral fibers were demonstrated in the present study and this is consistent with reports on rats, cats, and marsupials. Although the density of the ipsilateral fibers was much lower than that of the contralateral fibers, their distribution in the gray matter was similar to that of the contralateral fibers in the cervical and thoracic segments. These ipsilateral fibers were not seen in lumbar and lower segments.

It is concluded that the brain control of movement and homeostatic control is orchestrated by a large number of brain regions which correspond to most of those identified in the rat but include some known in cats and primates.
PUBLICATIONS ARISING FROM THIS THESIS

Journal articles


Conference abstracts

Huazheng Liang, George Paxinos, Charles Watson. Monosynaptic projections from the precuneiform area to the spinal cord of the mouse. ANS scientific meetings 2011, Auckland.

Huazheng Liang, George Paxinos, Charles Watson. Projections from the cuneiform complex to the spinal cord in the mouse. ANS scientific meetings 2009, Canberra.

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LIST OF ABBREVIATIONS

1Sp: lamina 1 of the spinal gray
2SpI: lamina 2 of the spinal gray, inner part
2SpO: lamina 2 of the spinal gray, outer part
3N: oculomotor nucleus
3Sp: lamina 3 of the spinal gray
3V: third ventricle
4N: trochlear nucleus
4Sp: lamina 4 of the spinal gray
4V: 4th ventricle
5n: trigeminal nerve
5N: motor trigeminal nucleus
5SpL: lamina 5 of the spinal gray, lateral part
5SpM: lamina 5 of the spinal gray, medial part
6N: abducens nucleus
6SpL: lamina 6 of the spinal gray, lateral part
6SpM: lamina 6 of the spinal gray, medial part
7n: facial nerve
7N: facial nucleus
7Sp: lamina 7 of the spinal gray
8n: vestibulocochlear nerve
8Sp: lamina 8 of the spinal gray
10N: dorsal motor nucleus of vagus
10Cb: lobule 10 of the cerebellar vermis
10Sp: area 10 of the spinal gray
12N: hypoglossal nucleus
A5: A5 noradrenaline cells
A8: A8 dopamine cells
A11: A11 dopamine cells
ac: anterior commissure
aca: anterior commissure, anterior part
acp: anterior commissure, posterior nerve
AHA: anterior hypothalamic area, anterior part
AHP: anterior hypothalamic area, posterior part
Amb: ambiguus nucleus
AmbC: ambiguus nucleus, compact part
AP: area postrema
Aq: aqueduct
Arc: arcuate hypothalamic nucleus
ArcL: arcuate hypothalamic nucleus, lateral part
Au1: primary auditory cortex
AuD: secondary auditory cortex, dorsal
AuV: secondary auditory cortex, ventral
Ax9: axial muscle motoneurons of lamina 9
Bar: Barrington’s nucleus
BDA: biotinylated dextran amine
Bi9: biceps motoneurons of lamina 9
BLA: basolateral amygdaloid nucleus, anterior part
Bo: Bötzinger complex
C1: C1 adrenaline cells
C1QL2: complement component 1, q subcomponent like 2
cc: corpus callosum
CC: central canal
CeCv: central cervical nucleus of the spinal cord
CeM: central amygdaloid nucleus, medial division
CEx9: crural extensor motoneurons of lamina 9
CFI9: crural flexor motoneurons of lamina 9
cg: cingulum
Cg1: cingulate cortex, area 1
CIC: central nucleus of the inferior colliculus
CMAd: dorsal part of the cingulate motor area
CMAr: rostral part of the cingulate motor area
CMAV: ventral part of the cingulate motor area
cp: cerebral peduncle
CPu: caudate putamen (striatum)
CnF: cuneiform nucleus
cu: cuneate fasciculus
Cu: cuneate nucleus
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CVL:</td>
<td>caudoventrolateral reticular nucleus</td>
</tr>
<tr>
<td>D3V:</td>
<td>dorsal 3rd ventricle</td>
</tr>
<tr>
<td>DA:</td>
<td>dorsal hypothalamic area</td>
</tr>
<tr>
<td>DAB:</td>
<td>3,3’-Diaminobenzidine</td>
</tr>
<tr>
<td>dcs:</td>
<td>dorsal corticospinal tract</td>
</tr>
<tr>
<td>De9:</td>
<td>deltoid muscle motoneurons of lamina 9</td>
</tr>
<tr>
<td>DI:</td>
<td>dysgranular insular cortex</td>
</tr>
<tr>
<td>Dk:</td>
<td>nucleus of Darkschewitsch</td>
</tr>
<tr>
<td>DLG:</td>
<td>dorsal lateral geniculate nucleus</td>
</tr>
<tr>
<td>DLPAG:</td>
<td>dorsolateral periaqueductal gray</td>
</tr>
<tr>
<td>DM:</td>
<td>dorsomedial hypothalamic nucleus</td>
</tr>
<tr>
<td>DMPAG:</td>
<td>dorsomedial periaqueductal gray</td>
</tr>
<tr>
<td>DMSp5:</td>
<td>dorsomedial spinal trigeminal nucleus</td>
</tr>
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<td>DMTg:</td>
<td>dorsomedial tegmental area</td>
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<tr>
<td>DpG:</td>
<td>deep gray layer of superior colliculus</td>
</tr>
<tr>
<td>DPGi:</td>
<td>dorsal paragigantocellular nucleus</td>
</tr>
<tr>
<td>DpWh:</td>
<td>deep white layer of superior colliculus</td>
</tr>
<tr>
<td>DR:</td>
<td>dorsal raphe nucleus</td>
</tr>
<tr>
<td>DRD:</td>
<td>dorsal raphe nucleus, dorsal part</td>
</tr>
<tr>
<td>DRL:</td>
<td>dorsal raphe nucleus, lateral part</td>
</tr>
<tr>
<td>DRV:</td>
<td>dorsal raphe nucleus, ventral part</td>
</tr>
<tr>
<td>EA:</td>
<td>extended amygdala</td>
</tr>
<tr>
<td>EAC:</td>
<td>extended amygdala, central part</td>
</tr>
<tr>
<td>ec:</td>
<td>external capsule</td>
</tr>
<tr>
<td>ECIC:</td>
<td>external cortex of the inferior colliculus</td>
</tr>
<tr>
<td>ECu:</td>
<td>external cuneate nucleus</td>
</tr>
<tr>
<td>ERS:</td>
<td>epirubrospinal nucleus</td>
</tr>
<tr>
<td>EW:</td>
<td>Edinger-Westphal nucleus</td>
</tr>
<tr>
<td>f:</td>
<td>fornix</td>
</tr>
<tr>
<td>FEx9:</td>
<td>forearm extensor motoneurons of lamina 9</td>
</tr>
<tr>
<td>FF19:</td>
<td>forearm flexor motoneurons of lamina 9</td>
</tr>
<tr>
<td>FG:</td>
<td>Fluoro-Gold</td>
</tr>
<tr>
<td>fi:</td>
<td>fimbria of the hippocampus</td>
</tr>
<tr>
<td>Fl:</td>
<td>flocculus</td>
</tr>
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</table>
fmi: forceps minor of the corpus callosum
fr: fasciculus retroflexus
GABA: \(\gamma\)-aminobutyric acid
GAD67: glutamic acid decarboxylase 67
Gi: gigantocellular reticular nucleus
GI: granular insular cortex
GiA: gigantocellular reticular nucleus, alpha part
GiV: gigantocellular reticular nucleus, ventral part
Gi9: gluteal motoneurons of lamina 9
GP: globus pallidus
gr: gracile fasciculus
Gr: gracile nucleus
H: H field of Forel
Hm9: hamstring motoneurons of lamina 9
HRP: horseradish peroxidase
IB: internal basilar nucleus
ic: internal capsule
IC: inferior colliculus
ICl: intercalated nucleus
ICo9: intercostal muscle motoneurons of lamina 9
icp: inferior cerebellar peduncle
IF: interfascicular nucleus
IH9: infrahyoid muscle motoneurons of lamina 9
ILL: intermediate nucleus of the lateral lemniscus
IML: intermedialateral column
IMM: intermedialmedial column
InC: interstitial nucleus of Cajal
InCSh: interstitial nucleus of Cajal, shell region
InG: intermediate gray layer of the superior colliculus
Int: interposed cerebellar nucleus
Int5: intertrigeminal nucleus
IntA: interposed cerebellar nucleus, anterior part
IntP: interposed cerebellar nucleus, posterior part
InWh: intermediate white layer of the superior colliculus
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tbody>
<tr>
<td>IO</td>
<td>inferior olivary nucleus</td>
</tr>
<tr>
<td>IOD</td>
<td>inferior olive, dorsal nucleus</td>
</tr>
<tr>
<td>IOM</td>
<td>inferior olive, medial nucleus</td>
</tr>
<tr>
<td>IOPr</td>
<td>inferior olive, principal nucleus</td>
</tr>
<tr>
<td>IP</td>
<td>interpeduncular nucleus</td>
</tr>
<tr>
<td>IPC</td>
<td>interpeduncular nucleus, caudal subnucleus</td>
</tr>
<tr>
<td>IPF</td>
<td>interpeduncular fossa</td>
</tr>
<tr>
<td>IRt</td>
<td>intermediate reticular nucleus</td>
</tr>
<tr>
<td>isRt</td>
<td>isthmic reticular formation</td>
</tr>
<tr>
<td>KF</td>
<td>Kölliker-Fuse nucleus</td>
</tr>
<tr>
<td>L4</td>
<td>4th lumbar segment</td>
</tr>
<tr>
<td>Lat</td>
<td>lateral cerebellar nucleus</td>
</tr>
<tr>
<td>LatC</td>
<td>lateral cervical nucleus</td>
</tr>
<tr>
<td>LC</td>
<td>locus coeruleus</td>
</tr>
<tr>
<td>LD9</td>
<td>latissimus dorsi motoneurons of lamina 9</td>
</tr>
<tr>
<td>LDTg</td>
<td>laterodorsal tegmental nucleus</td>
</tr>
<tr>
<td>Lfp</td>
<td>longitudinal fasciculus of pons</td>
</tr>
<tr>
<td>LH</td>
<td>lateral hypothalamic area</td>
</tr>
<tr>
<td>ll</td>
<td>lateral lemniscus</td>
</tr>
<tr>
<td>LPAG</td>
<td>lateral periaqueductal gray</td>
</tr>
<tr>
<td>LPB</td>
<td>lateral parabrachial nucleus</td>
</tr>
<tr>
<td>LPBC</td>
<td>lateral parabrachial nucleus, central part</td>
</tr>
<tr>
<td>LPBE</td>
<td>lateral parabrachial nucleus, external part</td>
</tr>
<tr>
<td>LPBV</td>
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</tr>
<tr>
<td>LPGi</td>
<td>lateral paragigantocellular nucleus</td>
</tr>
<tr>
<td>LPGiE</td>
<td>lateral paragigantocellular nucleus, external part</td>
</tr>
<tr>
<td>LPtA</td>
<td>lateral parietal association cortex</td>
</tr>
<tr>
<td>LRt</td>
<td>lateral reticular nucleus</td>
</tr>
<tr>
<td>LSO</td>
<td>lateral superior olive</td>
</tr>
<tr>
<td>LSp</td>
<td>lateral spinal nucleus</td>
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<tr>
<td>Lth</td>
<td>lithoid nucleus</td>
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<td>LV</td>
<td>lateral ventricle</td>
</tr>
<tr>
<td>LVe</td>
<td>lateral vestibular nucleus</td>
</tr>
<tr>
<td>m1</td>
<td>mesomere 1</td>
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</table>
M1: primary motor cortex
M2: secondary motor cortex
m5: motor root of the trigeminal nerve
MA3: medial accessory oculomotor nucleus
MCLH: magnocellular nucleus of the lateral hypothalamus
mcp: middle cerebellar peduncle
MCPC: magnocellular nucleus of the posterior commissure
MdD: medullary reticular nucleus, dorsal part
MdV: medullary reticular nucleus, ventral part
ME: median eminence
Me5: mesencephalic trigeminal nucleus
Med: medial cerebellar nucleus
MedDL: medial cerebellar nucleus, dorsolateral protuberance
MG: medial geniculate nucleus
MiTg: microcellular tegmental nucleus
ml: medial lemniscus
mlf: medial longitudinal fasciculus
MLR: mesencephalic locomotor region
MM: medial mammillary nucleus, medial part
MnR: median raphe nucleus
mp: mammillary peduncle
MPB: medial parabrachial nucleus
MPBE: medial parabrachial nucleus external part
MPL: medial paralemniscal nucleus
mRt: mesencephalic reticular formation
mt: mammillothalamic tract
MVe: medial vestibular nucleus
MVeMC: medial vestibular nucleus, magnocellular part
MVePC: medial vestibular nucleus, parvicellular part
ns: nigrostriatal tract
och: optic chiasm
Op: optic nerve layer of the superior colliculus
opt: optic tract
p1: prosomere 1
p1Rt: p1 reticular formation
Pa4: paratrochlear nucleus
PaAP: paraventricular hypothalamic nucleus, anterior parvicellular part
PAG: periaqueductal gray
PaMP: paraventricular hypothalamic nucleus, medial parvicellular part
PaPo: paraventricular hypothalamic nucleus, posterior part
PaR: pararubral nucleus
PaXi: paraxiphoid nucleus of thalamus
pc: posterior commissure
PCom: nucleus of the posterior commissure
PCRt: parvicellular reticular nucleus
PCRtA: parvicellular reticular nucleus, alpha part
Pe: periventricular hypothalamic nucleus
Pee9: pectoral muscle motoneurons of lamina 9
PeF: perifoncal nucleus
PeFLH: perifoncal part of lateral hypothalamus
PF: parafascicular thalamic nucleus
PFL: paraflocculus
PH: posterior hypothalamic nucleus
Ph9: phrenic motoneurons of lamina 9
PHA-L: phaseolus vulgaris leucoagglutinin
PL: paralemniscal nucleus
PLH: peduncular part of lateral hypothalamus
PMd: premotor area dorsal
PMv: premotor area ventral
PMn: paramedian reticular nucleus
Pn: pontine nuclei
PnC: pontine reticular nucleus, caudal part
PnO: pontine reticular nucleus, oral part
PnV: pontine reticular nucleus, ventral part
PPy: parapyramidal nucleus
Pr: prepositus nucleus
PR: prerubral field
Pr5DM: principal sensory trigeminal nucleus, dorsomedial part
Pr5VL: principal sensory trigeminal nucleus, ventrolateral part
PrBo: pre-Bötzinger complex and
PrCnF: precuneiform nucleus
PrEW: pre-Edinger-Westphal nucleus
psdc: postsynaptic dorsal column pathway
PSTh: parasubthalamic nucleus
PtA: parietal association cortex
PTg: pedunculotegmental nucleus
py: pyramidal tract
pyx: pyramidal decussation
RAmb: retroambiguus nucleus
RCh: retrochiasmatic area
RChL: retrochiasmatic area, lateral part
Rh9: rhomboid muscle motoneurons of lamina 9
RIP: raphe interpositus nucleus
RLi: rostral linear nucleus (midbrain)
RM: retromammillary nucleus
RMc: red nucleus, magnocellular part
RMg: raphe magnus nucleus
ROb: raphe obscurus nucleus
RPa: raphe pallidus nucleus
RPC: red nucleus, parvicellular part
RPF: retroparafascicular nucleus
RR: retrorubral nucleus
RRF: retrorubral field
rs: rubrospinal tract
Rt: reticular nucleus (prethalamus)
RtTg: reticulotegmental nucleus of the pons
RTz: retrotrapezoid nucleus
RVL: rostroventrolateral reticular nucleus
RVRG: rostral ventral respiratory group
S1: primary somatosensory cortex
S1BF: primary somatosensory cortex, barrel field
S1DZ: primary somatosensory cortex, dysgranular zone
S1FL: primary somatosensory cortex, forelimb region
S1HL: primary somatosensory cortex, hindlimb region
S1Sh: primary somatosensory cortex, shoulder region
S1Tr: primary somatosensory cortex, trunk region
S1ULp: primary somatosensory cortex, upper lip region
S2: secondary somatosensory cortex
s5: sensory root of the trigeminal nerve
SC: superior colliculus
SCh: suprachiasmatic nucleus
scp: superior cerebellar peduncle
SI9: supraspinatus and infraspinatus motoneurons of lamina 9
sm: stria medullaris
SMA: supplementary motor area
SMI-32: Neurofilament H non-phosphorylated
SNR: substantia nigra, reticular part
sol: solitary tract
Sol: nucleus of solitary tract
SolC: nucleus of solitary tract, commissural part
SolI: nucleus of solitary tract, interstitial part
SolL: nucleus of solitary tract, lateral part
SolM: nucleus of solitary tract, medial part
SolV: nucleus of solitary tract, ventral part
SolVL: nucleus of solitary tract, ventrolateral part
sp5: spinal trigeminal tract
Sp5C: caudal spinal trigeminal nucleus, caudal part
Sp5I: spinal trigeminal nucleus, interpolar part
Sp5O: spinal trigeminal nucleus, oral part
SPF: subparafascicular thalamic nucleus
SPO: superior paraolivary nucleus
SpVe: spinal vestibular nucleus
Sr9: serratus anterior motoneurons in lamina 9
st: stria terminalis
STh: subthalamic nucleus
STMPL: bed nucleus of the stria terminalis, medial division, posterolateral part
STMPM: bed nucleus of the stria terminalis, medial division, posteromedial part
Su3: supraoculomotor periaqueductal gray
Su5: supratrigeminal nucleus
Su3C: supraoculomotor cap
SubCA: subcoeruleus nucleus, alpha part
SubCD: subcoeruleus nucleus, dorsal part
SubCV: subcoeruleus nucleus, ventral part
SuG: superficial gray layer of the superior colliculus
SuVe: superior vestibular nucleus
T1: 1st thoracic segment
Te: terete hypothalamic nucleus
TeA: temporal associating cortex
TMB: 3,3′,5,5′-tetramethylbenzidine
Tr9: triceps motoneurons of lamina 9
TrLL: triangular nucleus, lateral lemniscus
ts: tectospinal tract
TuLH: tuberal region of lateral hypothalamus
Tz: nucleus of the trapezoid body
TzSM9: trap/sternom motoneurons of lamina 9
V1: primary visual cortex
V2: secondary visual cortex
V2MM: secondary visual cortex, mediomed
VCA: ventral cochlear nucleus, anterior part
VeCb: vestibulocerebellar nucleus
VLL: ventral nucleus of the lateral lemniscus
VLPAG: ventrolateral periaqueductal gray
vmf: ventral median fissure
VMH: ventromedial hypothalamic nucleus
VMHC: ventromedial hypothalamic nucleus, central part
VMHDM: ventromedial hypothalamic nucleus, dorsomedial part
VMHSh: ventromedial hypothalamic nucleus, shell region
VMHVL: ventromedial hypothalamic nucleus, ventrolateral part
VPM: ventral posteromedial thalamic nucleus
VTA: ventral tegmental area
vwc: ventral white commissure
WGA-HRP: wheat germ agglutinin-horseradish peroxidase
Xi: xiphoid thalamic nucleus
xscp: decussation of the superior cerebellar peduncle
YFP: yellow fluorescent protein
ZID: zona incerta, dorsal part
CHAPTER I. General introduction
Descending projections from the brain to the spinal cord have been widely studied in a variety of species. While there is a high degree of similarity across species in the organization of spinal projecting neuronal groups, differences between species are observed. This review concerns itself with the projections from the brain to the spinal cord and will discuss the spinal projecting neuronal groups in mammals which have been extensively studied (mouse, rat, cat, monkey).

1.1 Prosencephalon

1.1.1 Telencephalon

1.1.1.1 Cerebral cortex
The cerebral cortex has been well studied in relation to locomotion (Liddell and Phillips, 1944; Adkins et al., 1971; Orlovsky 1972; Eidelberg and Yu, 1981; Armstrong and Drew, 1984; 1985), grasping (in monkeys: Rizzolatti et al., 1988; Sakata et al., 1995; Murata et al., 1997; Murata et al., 2000; Raos et al., 2006), reaching (in humans: Cavina-Pratesi et al., 2010; in monkeys: Fattori et al., 2010), perception (in rats: Welker 1971; Sapienza et al., 1981; Dawson and Killackey, 1987; Li et al., 1990), vision (in rats: La Messurier 1948; Montero 1973; Vargo et al., 1988), audition (in dogs: Tunturi 1952; in cats: Merzenich et al., 1975; Reale and Imig, 1980). The following section will review the corticospinal projections in four mammal species: the mouse, rat, cat, and the monkey.

In the mouse (anatomy of mouse cerebral cortex shown in Fig 1.1, 2, and 3. All sections and diagrams are from “Franklin KBJ and Paxinos G. The mouse brain in stereotaxic coordinates. 3rd edition, Elsevier, Academic Press, San Diego), some studies have investigated the projections from the cerebral cortex by injecting retrograde tracers to the spinal cord. In the study of injecting retrograde tracers – adenovirus and horseradish peroxidase (HRP) into the upper cervical cord of the mouse, labeled neurons are found in the motor cortex (Tsukamoto et al., 2003). Another study reported that labeled neurons are found in the sensorimotor cortex, lateral agranular cortex, medial part of the granular cortex, rostral part of the agranular cortex, the secondary somatosensory cortex, the visual cortex, and parietal areas (Sbriccoli et al., 1995). Anterograde studies with biotin conjugated dextran amine and DiI have verified the sensorimotor-spinal cord projections (Steward et al., 2004; Sibbe et al., 2007). In Thy-1-YFP mice, corticospinal neurons labeled by YFP are observed mainly in layer 5 and in a smaller percentage in layer 3. There are two possibilities to explain the
corticospinal neurons found in layer 3. (a) The tracer injection in the present and other studies may not be as sensitive as genetic labelling. (b) The Thy-1-YFP might label some neurons other than corticospinal neurons and they are false positive. Corticospinal fibers can be clearly seen in the way though the medulla and the spinal cord. Furthermore, it has been shown that the mouse has corticospinal fibers not only in the dorsal column, but also in the dorsolateral column, the ventral column, and the lateral-most white matter. The dorsal corticospinal tract, which contains about 90% of the total corticospinal fibers, contains bifurcating fibers projecting to both the dorsal horn and the intermediate laminae. These fibers are thin in diameter, whereas those in the dorsolateral and the ventral tracts are thicker and many terminate on motor neurons. The monosynaptic projections to the spinal cord motoneurons are from the dorsolateral and ventral corticospinal tracts. These have been confirmed with retrograde tracer injection to the hindlimb muscles of Thy-1-YFP mice (Bareyre et al., 2005).

In the rat, the distribution of corticospinal neurons is similar to that of the mouse. A large number of corticospinal neurons are labeled in the motor cortical areas (primary and

Fig I.1 A coronal section at the level of the rostral pole of the mouse thalamus showing the structure of the cortex and the bed nucleus of stria terminalis. The photograph on the left is an AChE stained section, the diagram on the right is the corresponding drawing.
secondary motor cortex) after spinal cord injections of the retrograde tracer (Schwanzel-Fukuda et al., 1984; Miller 1987; Masson et al., 1991; Nudo et al., 1995).

After upper cervical injections of the retrograde tracer, it can be seen that the majority of labeled neurons are located in layer 5b, with a small number of cells scattered in layers 5a and 5c. In area 4 (corresponding to primary motor cortex), corticospinal neurons are observed throughout the rostrocaudal extent, but the density of labeled neurons drops dramatically in the rostral pole. In area 6 (corresponding to the secondary motor cortex), labeled neurons can only be observed in the rostral one third (Miller 1987). Corticospinal neurons are also observed in the medial primary and secondary somatosensory cortex. In the primary somatosensory area, corticospinal neurons seem to be continuous with those in the primary motor cortex. They are consistently observed in the limb representing areas, to a lesser extent in the shoulder representing area and the dysgranular somatosensory area. In the secondary somatosensory area, corticospinal neurons are observed mainly at caudal levels. Compared to motor cortical areas, there are fewer layers of corticospinal neurons in somatosensory cortical areas. In the motor and somatosensory cortical areas, labeled neurons in layer 5 constitute about 48% of total layer 5 neurons. In addition, corticospinal neurons are observed in the posterior parietal (areas 14, 39, 40), occipital (areas 18a, 18b), cingulate (areas 24a, 24b) and prefrontal (area 32) cortex (Miller 1987). But another study shows that corticospinal neurons in the occipital area appear only for two weeks after birth. These neurons are smaller in size and not consistently observed as in primary motor and primary somatosensory areas (Stanfield and O’Leary, 1985).

In contrast to the upper cervical injection results, the upper thoracic cord injection of the retrograde tracer labels corticospinal neurons in the more rostral part of the secondary motor cortex (compared with those labeled neurons after cervical injections), scattered corticospinal neurons in the limb representing areas of the primary somatosensory cortex, clustered corticospinal neurons in the caudal half of the secondary somatosensory cortex. Though labeled corticospinal neurons are observed in the posterior parietal (areas 14, 39, 40), occipital (areas 18a, 18b), cingulate (areas 24a, 24b), and the prefrontal area (area 32), there are fewer neurons labeled following the thoracic injections of the retrograde tracer. There are even fewer neurons labeled in the above mentioned areas after lower thoracic injections of the retrograde tracer and the majority of them are located in the dorsomedial part of these areas (Miller 1987).
Unlike cortical labelling following cervical and thoracic injections, lumbar injections of the retrograde tracer reveal that labeled neurons are restricted to a caudal stripe situated along the primary motor and primary somatosensory cortex border and in the anteromedial part of the occipital cortex (visual cortex) (Miller 1987). Sacrocaudal injections, however, only label some neurons in layer 5 of the motor cortex near the midline (Masson et al., 1991).

Corticospinal tract (cst) fibers in the rat spinal cord occupy a position similar to that occupied in the mouse spinal cord. The majority of the corticospinal fibers are seen in the contralateral dorsal column, with a small number of fiber bundles in the ipsilateral ventral funiculus (only at cervical and upper thoracic levels) and the contralateral dorsolateral column (Casale et al., 1988; Joosten et al., 1992). In the gray matter of the spinal cord, most of the corticospinal fibers terminate in the medial portion of the contralateral laminae 3-6, and to a lesser extent in lamina 7. Sparse fibers are also seen in laminae 8, 9 and 10. This distribution pattern is similar to that of the mouse, but Joosten et al (1992) reported that the ipsilateral fibers go through a developmental change in postnatal stages. In 2 weeks after birth, these ipsilateral fibers can not be seen below upper thoracic segments. However, Brosamle and Schwab (1997 and 2000) demonstrated that the ipsilateral fibers travel down to lumbar segments and they are mainly distributed in laminae 3-6. After birth, the corticospinal fibers go through the elimination from the ventrolateral portion of the spinal cord gray matter. This starts on the 7th postnatal day and stops on the 11th postnatal day (Kamiyama et al., 2006). Unlike the fiber distribution pattern seen in the transgenic mice, there are no fiber bundles in the lateral most gray matter in the rat, a difference that might be explained by the limitation of traditional injecting methods.

In the cat, corticospinal neurons are principally found in the contralateral primary (area 4) and secondary motor cortex (area 6). They consist of large pyramidal neurons in layer 5 and form clusters of 5 to 10 neurons in a columnar pattern. The primary somatosensory cortex (3b, 1, 2) and the transition zone (3a) between the primary motor and primary somatosensory cortex also have a considerable number of corticospinal neurons. Fewer corticospinal neurons are found in the secondary somatosensory cortex (area 2 Pri). As in the rat, there is also a topographic organization of the corticospinal neurons (cervical projecting neurons are more laterally located and lumbar projecting neurons are more medially located) (Armand et al.,
Fig. 1.2 A coronal section at the level of the caudal paraventricular nucleus showing the structure of the cortex, amygdala, and nuclei in the hypothalamus. The photograph on the left is a Nissl stained section, the diagram on the right is the corresponding drawing.

1974; Armand and Aurenty, 1977; Groos et al., 1978; Hayes and Rustioni, 1981; Keizer and Kuypers, 1984; Ghosh 1997). Consistent with results in the mouse and rat, corticospinal neurons are observed from the medial wall of the hemisphere to the lateral part of the cortex with tightly packed neurons in the primary motor cortex.

The corticospinal tract in the cat travels in the lateral and ventral funiculi, with the majority of its fibers in the lateral funiculus (Nyberg-Hansen and Brodal, 1963; Nyberg-Hansen 1969; Cheema et al., 1984; Armand et al., 1985), and this distribution pattern is different from that of the mouse and rat. The presence of the dorsal corticospinal tract in the dorsal column has been reported, but its fiber distribution is difficult to distinguish from other tracts (Satomi et al., 1989). Both contralateral and ipsilateral fibers are seen in the spinal cord and they travel down to lower lumbar or sacral segments (Nyberg-Hansen and Brodal, 1963; Nyberg-Hansen 1969; Satomi et al., 1989). Furthermore, differences in the fiber distribution of different cortical areas in the spinal cord have been observed. Fibers from the lateral gyrus proprius (frontal cortex), the medial orbitofrontal cortex, and the secondary somatosensory area travel in the bilateral lateral funiculus and reach the sacral segment. There are fewer
fibers from somatosensory areas than from motor areas. The parietal cortex has a different terminating pattern. Only a few fibers are seen in the contralateral lateral funiculus and they mainly terminate on cervical segments with scattered fibers in lumbosacral segments (Nyberg-Hansen 1969). Dense fibers on the contralateral side enter the gray matter from the lateral part of laminae 5 and 6 and radiate in a fan shape to laminae 4 to 7 (Nyberg-Hansen 1966; 1969). Interestingly, fibers from the primary motor cortex occupy both the dorsomedial and ventromedial parts of the lateral corticospinal tract, whereas fibers from the primary somatosensory cortex only occupy the ventromedial part of the lateral corticospinal tract (Nyberg-Hansen 1966). However, ipsilateral fibers are mainly restricted to laminae 7 and 8 (Cheema et al., 1984). In addition, some fibers are seen in laminae 1 and 2 in C7/8, T1, L4-7 on the contralateral side (Cheema et al., 1984; Armand et al., 1985). A small number of fibers are also present in the dorsolateral part of lamina 9 of some segments (Cheema et al., 1984). Physiological recording and tracer injection studies allow the possibility of monosynaptic projections from the cortex to spinal cord motor neurons but do not confirm these projections (Rikard-Bell et al., 1985; Rikard-Bell et al., 1986). This fiber distribution pattern in the gray matter is similar to that of the mouse and rat.

A further study revealed that the dorsolateral corticospinal tract is composed of crossed and uncrossed fibers with a ratio of 10:1, whereas the ventral corticospinal tract is composed of crossed and uncrossed fibers with a ratio of 1:1. By injecting a retrograde tracer and transecting the spinal cord rostral to the injection site, the primary motor cortex is found to give rise to bilateral corticospinal fibers in both the dorsolateral and ventral corticospinal tracts. In contrast, the primary and secondary somatosensory cortices predominantly give rise to the contralateral fibers (Armand and Kuypers, 1980).

In the monkey, the primary motor cortex is also the major source of corticospinal fibers. The precentral area 4 (the primary motor cortex), which contains the forelimb and hindlimb representations, has the largest number of spinal projecting neurons (Hutchins et al., 1988; Nudo and Masterton, 1990; Dum and Strick, 1991; He et al., 1995). In the medial wall of the frontal lobe, another 3 areas also have corticospinal neurons. The first location is the supplementary motor area (SMA), which is located in the medial part of area 6 (the secondary motor cortex) at the level caudal to the posterior arcuate sulcus. The second location is the cingulate motor area in region 23c at the intermediate level of the cingulate sulcus rostral to the boundary of area 6/4. Corticospinal neurons are present in both the dorsal
(CMAd) and ventral parts (CMAv) of the cingulate motor area. The third location is the rostral part of the cingulate motor area in region 24c (CMAr). Its corticospinal neurons mainly occupy the ventral bank of the cingulate sulcus. Corticospinal neurons in the SMA, CMAd, and CMAv are topographically positioned as in the primary motor cortex (Hutchins et al., 1988; Dum and Strick, 1991; He et al., 1995). In the lateral frontal lobe, there are another 2 areas, caudal to the border of area 4/6, which have corticospinal neurons. The first group is located in the caudal stripe of area 6 (named premotor area dorsal, PMd) and the other group is located in a more ventral position of area 6 (named premotor area ventral, PMv) (Dum and Strick, 1991; He et al., 1993). The PMv mainly projects to the upper cervical cord (Wise 2006), with some projections to the reticular formation of the brainstem, through which PMv establishes disynaptic connections with the spinal cord (Catsman-Berrevoets and Kuypers, 1976). Like the primary motor cortex, the PMd also has a topographic organization of its corticospinal neurons. Though there is some overlap between upper and lower cervical projecting neurons, they are separated from thoracic and lumbar projecting neurons (He et al., 1993).

Fig 1.3 A coronal section at the level of the subthalamic nucleus showing the structure of the cortex, amygdala, dorsal zona incerta, subthalamic nucleus, and nuclei in the hypothalamus. The photograph on the left is a Nissl stained section, the diagram on the right is the corresponding drawing.
The corticospinal tract principally travels in the dorsolateral funiculus of the spinal cord in the monkey, and its fibers enter the gray matter from the lateral part of laminae 5 and 6, radiating to other laminae. Generally, all motor areas have similar terminating patterns but slight differences also exist.

The primary motor cortex gives off bilateral fibers to the spinal cord. Among these fibers, 82% are contralateral and 70% of these contralateral fibers terminate in the intermediate zone with 28% terminating in lamina 9 (mainly in the lower cervical segments) and about 2% in the dorsal horn. Ipsilateral fibers constitute only about 18% of spinal projections and the majority of them terminate in the medial lamina 7 and 8 (Dum and Strick, 1996; Borra et al., 2010). This is similar to the finding in the mouse (Bareyre et al., 2005), rat (Casale et al., 1988), and the cat (Cheema et al., 1984). Furthermore, the density of fibers in the ipsilateral lamina 8 is similar to that of the contralateral side.

The supplementary motor area also gives off bilateral fibers to the spinal cord with a contralateral predominance (about 77%). On the contralateral side, the densest terminations are in the intermediate zone of the cervical cord (laminae 5–8) (approximately 87% of the total contralateral fibers), followed by the ventral horn (11%) and the dorsal horn (2%). Ipsilateral fibers comprise about 23% of the total corticospinal fibers. Most fibers terminate in lamina 8 and in the medial portion of lamina 7. In other areas, terminals are sparse.

The CMAd and CMAv send a higher percentage of contralateral fibers to the spinal cord compared to the primary and supplementary motor areas (91% of the total fibers). Among these contralateral fibers, 90% terminate in the intermediate zone and 4% in the ventral horn, and fibers are also seen in the dorsal horn. Among the fiber terminals in the intermediate zone, those from the CMAd concentrate in the dorsolateral portion of the intermediate zone, whereas those from the CMAv concentrate in the dorsomedial part of the intermediate zone. The density of fiber terminals from the CMAd and CMAv is lower than that of the SMA. Ipsilaterally, the descending fibers also concentrate in the medial lamina 7 and lamina 8 (Dum and Strick 1996).

In the monkey at birth, the corticospinal fibers have arrived at each segment of the spinal cord in the white matter, but the corticomotor connections (the corticospinal fibers
terminating on motor neurons in the spinal cord) haven’t been established. In the following 5 months, these connections begin to increase and plateau in the second year. These connections mature in a caudorostral direction in the cervical cord (Armand et al., 1997).

1.1.1.2 Amygdala
In the cat and monkey, spinal projections from the amygdala have also been reported. These spinal projections originate from the caudal portion of the central nucleus and travel down to the ipsilateral upper and middle cervical cord (Mizuno et al., 1985; Sandrew et al., 1986) (the anatomy of the mouse amygdala shown in Fig.12 and 3). In the cat, the medial nucleus also has a small number of spinal projecting neurons (Sandrew et al., 1986). However, in the rat, Schwanzel-Fukuda et al reported that the rostral amygdala is labeled after injecting the retrograde tracer to the caudal medulla (1984). This projection has not been reported in the mouse.

1.1.1.3 The bed nucleus of the stria terminalis
This nucleus may be involved in conditioning related to the startle response in experimental animals (in rats: Lee and Davis 1997; Walker and Davis 1997; Gewirtz et al., 1998) and mediate fear reactions (in rats: Wallace and Rosen 2001; Fendt et al., 2002). It has a small number of spinal projecting neurons in its medial and lateral parts in the rat (anatomy of this nucleus of the mouse shown in Fig.11). Descending fibers from this nucleus reach the lumbar cord (Schwanzel-Fukuda et al., 1984). In the cat, this nucleus has spinal projections as well, but these projections are only confined to the 1st cervical segment (Holstege et al., 1985). The fibers might originate from the medial or lateral part of the bed nucleus of the stria terminalis because the anterograde tracer covered both areas in the experimental cat. This has not been reported in the mouse and monkey.

1.1.2 Hypothalamus
The hypothalamus receives a wide range of inputs including olfactory (in rats: Price et al., 1991), visual (in humans: Sadun et a., 1984; Dai et al., 1998), somatosensory (in rats: Burstein et al., 1987; Cliffer et al., 1991), visceral sensory (in rats: Ricardo and Koh 1978; in monkeys: Beckstead et al., 1980) and modulates autonomic (in rats: Strack et al., 1989; Spencer et al., 1990; Jansen et al., 1992; Haxhiu et al., 1993; Schramm et al., 1993; Smith et al., 1998; in humans: Bamshad et al., 1982; in humans: Pelletier et al., 1983; Bloch et al., 1984; Mouri et al., 1984; Pelletier et al., 1986; Koutcherov et al., 2000), and behavioural (in rats: Hardy and Boulan 1974; in monkeys:
Hori et al., 1987; Szymusiak et al., 1998; Griffin and Boulant 1995; in humans: Rance et al., 1994; Tataranni et al., 1999; Gordon et al., 2000) function to maintain the homeostasis.

1.1.2.1 The paraventricular hypothalamic nucleus

This nucleus is the major source of hypothalamic projections to the spinal cord (anatomy of this nucleus of the mouse shown in Fig.12). This nucleus has bilateral spinal projections from both the magnocellular and parvicellular parts with an ipsilateral predominance in rats (Zemlan et al., 1979; Swanson and Kuypers, 1980a; Watkins et al., 1981; Sawchenko and Swanson, 1982; Leong et al., 1984a; 1984b; Schwanzel-Fukuda et al., 1984; Masson et al., 1991; Kudo et al., 1993; Hallbeck and Blomqvist, 1999; Hallbeck 2000; Pyner and Coote 2000; Puder and Papka, 2001; Kc et al. 2002; Condes-Lara et al., 2007), cats (Basbaum and Fields, 1979; Miura et al., 1983; Caverson et al., 1984; Holstege 1987a; Kausz 1990), and monkeys (Saper et al., 1976; Kneisley et al., 1978). Fibers from this nucleus reach the lumbosacral cord (Leong et al., 1984a). In the magnocellular part of this nucleus, there are oxytocin and vasopressin reactive spinal projecting neurons. In the rat, vasopressin reactive neurons are concentrated posterodorsolaterally, whereas oxytocin reactive cells are localized anteroventromedially (Sawchenko and Swanson 1982). Oxytocinergic fibers reach the lumbar

![Fig1.4 A coronal section at the level of the dorsal lateral geniculate nucleus showing the structure of the cortex and nuclei of the diencephalon and hypothalamus. The photograph on the left is an AChE stained section, the diagram on the right is the corresponding drawing.](image-url)
A coronal section at the level of the medial geniculate nucleus showing the structure of nuclei in the diencephalon. The photograph on the left is an AChE stained section, the diagram on the right is the corresponding drawing.

cord and terminate on uterine and cervix related preganglionic neurons in the rat (Condes-Lara et al., 2007; Puder and Papka, 2001). Vasopressinergic fibers terminate on both the pre-Bötzinger complex and the spinal cord, relaying signals to respiratory motor neurons (Kc et al., 2002). Dynorphin reactive spinal projecting neurons are also found in the magnocellular part of the paraventricular nucleus in the rat (Hallbeck 2000).

The parvicellular part of the paraventricular nucleus in the rat also has oxytocin and vasopressin reactive spinal projecting neurons. The majority of these double labeled neurons (oxytocin and retrograde tracer double labeled neurons or vasopressin and retrograde tracer double labeled neurons) are in the caudal portion. In the posterior magnocellular and in the medial and lateral parvicellular parts of this nucleus, there are a few labeled neurons which are reactive to methionine-enkephalin. But few neurons are reactive to somatostatin (Sawchenko and Swanson 1982). Spinal projecting neurons in the midrostral portion of the paraventricular hypothalamic nucleus in the rat have collaterals to the rostroventrolateral reticular nucleus (RVL), suggesting that this nucleus plays a role in cardiovascular control (Pyner and Coote, 2000). In thoracic segments of the rat, cat, and the monkey, fibers from the
paraventricular nucleus are seen to terminate on the intermediolateral cell column (anatomy of mouse spinal cord shown in Fig. 1.15). This indicates a direct involvement of this nucleus in the modulation of the autonomic system (Saper et al., 1976). In the cat, its fibers also terminate on the intermediolateral cell column in lumbar and sacral segments and the nucleus of Onuf (Holstege 1987a). In the mouse, spinal projections from this nucleus have not been investigated.

1.1.2.2 The lateral hypothalamus

The lateral hypothalamus (LH) has spinal projections across species studied (anatomy of mouse LH shown in Fig. 2.4). These spinal projecting neurons extend from the level of the paraventricular nucleus medially, medial to the internal capsule laterally, to the H field of Forel and the medial part of the zona incerta dorsally at caudal levels in the rat, where labeled neurons are medial to the mammillothalamic tract (Zemlan et al., 1979; Leong et al., 1984a). Neurons in the LH are bilaterally labeled with an ipsilateral predominance (in rats: Swanson and Kuypers, 1980b; Leong et al., 1984a; in cats: Basbaum and Fields 1979; Holstege 1987; in monkeys: Kneisley et al., 1978). Among these neurons, some are hypocretin reactive. Their fibers can reach the sacral segment and are distributed in lamina 1, intermediolateral cell column, and lamina 10 (van den Pol 1999). In the ventral part of the rat hypothalamus, some spinal projecting neurons are also present in the retrochiasmatic nucleus (RCh), the ventral part of the ventromedial hypothalamic nucleus (VMH), and a few cells in the arcuate hypothalamic nucleus (Arc) and the suprachiasmatic nucleus (Sch). Their fibers reach lumbosacral segments (Swanson and Kuypers, 1980a; Leong et al., 1984a; Schwanzel-Fukuda et al., 1984; Masson et al., 1991). Projections from this nucleus to the spinal cord have not been investigated in the mouse.

1.1.2.3 The posterior hypothalamus

The posterior hypothalamus (PH) has a number of spinal projecting neurons whose fibers reach the sacral segment in the rat, but the number of labeled neurons drops after more caudal spinal cord injections (Schwanzel-Fukuda et al., 1984) (anatomy of mouse PH shown in Fig. 1.4). This nucleus has not been reported to project to the spinal cord in the mouse.

1.1.2.4 The subthalamic nucleus

This nucleus has been the target of deep brain stimulation for the treatment of Parkinson’s disease (in humans: Rodríguez et al., 1998; Francois et al., 2000; Stefani et al., 2007).
In the Japanese monkey, it is reported to project to the upper cervical cord (anatomy of the mouse counterpart shown in Fig.1.3). The majority of these spinal projecting neurons are located in the rostral and dorsal parts of the subthalamic nucleus with a contralateral predominance (Mizuno et al., 1988). There is no similar report in the mouse.

1.1.3 Diencephalon

1.1.3.1 The dorsal zona incerta

The dorsal zona incerta (ZID) has strongly labeled spinal projecting neurons, mostly located in the medial portion of this nucleus on the ipsilateral side in the rat (Basbaum and Fields, 1979; Leong et al., 1984a; Schwanzel-Fukuda et al., 1984) (anatomy of this nucleus of the mouse shown in Fig.1.3 and 4). In the cat and monkey, spinal projecting neurons are also observed after tracer injections to the spinal cord, but the number of labeled neurons is smaller than that of the rat (Nudo and Masterton, 1988). Though these labeled neurons are in a similar location to that of the A13 neurons, they are not dopaminergic (Skagerberg and Lindvall, 1985).

1.1.3.2 A11
This nucleus is the only dopaminergic nucleus in the diencephalon which has spinal projecting neurons. In the mouse, so far there has been only one study on diencephalic projections to the spinal cord (Qu et al., 2006) (anatomy of mouse A11 shown in Fig.1.4). This study demonstrated that A11 has dopaminergic spinal projecting neurons, and that these neurons are located close to the midline and dorsal to the third ventricle. In the rat, A11 has similar projections to the spinal cord and approximately half of these projecting neurons are catecholaminergic (Björklund and Skagerberg, 1979). Though there are reports that the subparafascicular thalamic nucleus in the rat has dopaminergic neurons projecting to the spinal cord, these cells might belong to the A11 group (Morizumi and Hattori, 1992; Takada 1993).
1.1.3.3 H fields of Forel

The H field of Forel (H) is involved in head movement through its connections with motoneurons in the cervical cord (in cats: Isa et al., 1988; Isa and Sasaki 1992a; 1992b). It has fusiform shaped cells throughout its whole extent projecting to the lumbar cord but they are only loosely packed in the rat (Leong et al., 1984a) (anatomy of this nucleus of the mouse shown in Fig 1.4). However in the cat this nucleus has more labeled neurons after cervical injections, and it has monosynaptic projections to the neck motor neurons (Isa et al., 1988; Holstege and Cowie, 1989; Isa and Sasaki, 1992a; 1992b). Additionally in the cat, fibers from this nucleus can be seen in laminae 5 to 8 in the upper cervical cord (Isa and Sasaki, 1992a). Holstege and Cowie (1989) found that fibers from this nucleus mainly terminate in the lateral ventral horn in the upper cervical cord and in the medial ventral horn in the lower cervical cord. In the thoracic and lumbar cord, fibers are very sparse. In the mouse and monkey, this nucleus has not been reported to have spinal projections.

1.1.3.4 The parafascicular nucleus

The parafascicular nucleus (PF) is associated with pain processing (Weigel and Krauss 2004) and attention (Raeva et al., 2006). Deep stimulation studies in humans show that his nucleus is involved in hyperkinetics disorders (Vandewalle et al., 1999; Maciunas et al., 2007; Welter et al., 2008). It contains spinal projecting neurons in its lateral part in the rat (anatomy of this nucleus of the mouse shown in Fig 1.4). These neurons give off descending fibers to the contralateral spinal cord which travel in the ventrolateral funiculus and terminate in the ventral horn. These fibers can only be seen in the cervical and thoracic cord (Marini et al. 1999). In other species this nucleus has not been reported to have spinal projections.

1.1.3.5 The interstitial nucleus of Cajal

The interstitial nucleus of Cajal (InC) is related to vertical gaze holding and eye-head coordination (in monkeys: Westheimer and Blair 1975; Fukushima 1987 review). It has bilateral spinal projections with an ipsilateral predominance in the rat (anatomy of mouse InC shown in Fig 1.5 and 6). Its fibers travel down to the lumbar cord (Leong et al., 1984a). But Zemalan et al (1979) only found labeled cells in this nucleus after upper cervical injections. In the cat (Nyberg-Hansen, 1966; Kuypers and Maisky, 1975; Holstege and Tan, 1988; Holstege and Cowie, 1989; Isa and Sasaki, 1992a; Satoda et al., 2002) and monkey (Castiglioni et al., 1978; Carlton et al., 1985; Nudo and Masterton, 1988), this nucleus has similar projections to the spinal cord as in the rat. Fibers from this nucleus reach the lumbar cord and mainly terminate in the ipsilateral cord via the dorsal part of the ventral funiculus in
cats (Nyberg-Hansen, 1966) and the dorsolateral funiculus in monkeys (Carlton et al., 1985). In the mouse, spinal projections from this nucleus have not been studied.

1.1.3.6 The nucleus of Darkschewitsch
The nucleus of Darkschewitsch (Dk) is the accessory oculomotor nucleus which is involved in papillary light reflex (in humans: Bianchi and Gioia 1990; Horn and Adameczyk, 2012; in rats: Klooster and Vrensen 1998). It contains a small number of ipsilaterally spinal projecting neurons in the rat (Leong et al., 1984a; 1984b; Lakkae 1997), cat (Holstege and Tan, 1988; Isa and Sasaki, 1992a), and the monkey (Castiglioni et al., 1978; Carlton et al., 1985; Nudo and Masterton, 1988) (anatomy of mouse Dk shown in Fig1.5 and 6). Descending fibers from this nucleus mainly travel in the dorsolateral funiculus in the monkey (Carlton et al., 1985). This projection is unknown in the mouse.

1.1.3.7 The nucleus of posterior commissure
The nucleus of posterior commissure (PCom) has a small number of spinal projecting neurons (in rats: Leong et al., 1984a; in cats: Spence and Saint-Cyr, 1988; Holstege and Cowie, 1989; Warren et al., 2008; in monkeys: Castiglioni et al., 1978) (anatomy of mouse PCom shown in Fig1.5). These neurons are bilaterally labeled in rats (Leong et al., 1984a) and cats (Holstege and Cowie, 1989; Warren et al., 2008), with an ipsilateral predominance, and their fibers mainly terminate in the medial part of ventral horn via the dorsal part of the ventral funiculus (in cats: Holstege and Cowie, 1989). Projections from this nucleus have not been studied in the mouse.

1.2. Mesencephalon

1.2.1 The red nucleus
This nucleus is involved in hand and finger movements (in monkeys: Lawrence and Kuypers 1968a; Gibson et al., 1985a; van Kan and McCurdy 2001) and, to a lesser extent in general limb movements such as scratching and locomotion (in rats: Muir and Whishaw 2000). It is also associated with antinociceptive function (in rats: Prado and Roberts 1985; Kumar et al., 1995).

The red nucleus has a large number of fibers descending to the contralateral spinal cord in all species studied (anatomy of mouse red nucleus shown in Fig1.5 and 6). These fibers originate not only from the magnocellular part (RMC) (In mice: Carretta et al., 2001; Tsukamoto et al., 2003; VanderHorst and Ulfhake, 2006; in rats: Leichnetz et al., 1978; Murray and Gurule,
179; Zemlan et al., 1979; Watkins et al., 1981; Shiel et al., 1983; Leong et al., 1984a; Schwanzel-Fukuda et al., 1984; Daniel et al., 1987; Shen et al., 1990; Masson et al., 1991; Kudo et al., 1993; Naso et al., 1993; Wang et al., 1996; de Boer-van Huizen and ten Donkelaar, 1999; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979; Holstege and Tan, 1988; Satoda et al., 2002; in monkeys: Castiglioni et al., 1978; Kneisley et al., 1978; Carlton et al., 1985) but also from the parvicellular part of the red nucleus (RPC) (In rats: Leong et al., 1984a; Daniel et al., 1987; In cats: Pong et al., 2002). Most of these neurons, however, are located in the RMC, with a smaller number of neurons in the RPC. There is a topographic organization of these spinal projecting neurons in the RMC. The cervical projecting neurons are located in the dorsomedial portion, and the lumbosacral projecting neurons in the ventrolateral portion (in mice: Carretta et al., 2001; in rats: Watkins et al., 1981; Leong et al., 1984; Daniel et al., 1987; in cats: Pompeiano and Brodal, 1957; Hayes and Rustioni, 1981; Holstege and Tan, 1988; Pong et al., 2002; in monkeys: Castiglioni et al., 1978; Kneisley et al., 1978). In the rat, the ventrolateral RMC has been clearly demarcated and retrogradely labeled from E18.5. In E20.5 rat embryos, labeled neurons can be observed in the caudoventrolateral and the rostroventral parts of the RMC (Kudo et al., 1993).

The rubrospinal tract principally travels in the contralateral dorsolateral funiculus in all species studied (in rats: Brown 1974; Leichnetz et al., 1978; Zemlan et al., 1979; Watkins et al., 1981; Antal et al., 1992; Yasui et al., 2001; Küchler et al., 2002; in cats: Gibson et al., 1984; McCurdy et al., 1987; Holstege 1987b; Fujito and Aoki, 1995; in monkeys: Miller and Strominger, 1973; Carlton et al., 1985), with a small number of fibers seen in the ipsilateral dorsolateral funiculus (in rats: Antal et al., 1992; in cats: Holstege 1987b). This tract reaches the lumbosacral segment. In the gray matter of the contralateral spinal cord, rubrospinal fibers terminate mainly in laminae 5, 6, and the dorsal part of lamina 7 (in rats: Brown 1974; Antal et al., 1992; Yasui et al., 2001; Küchler et al., 2002; in cats: Gibson et al., 1984; McCurdy et al., 1987; Holstege and Tan, 1988; in monkeys: Miller and Strominger, 1973; Ralston et al., 1988). Furthermore, some fibers also terminate on the dorsolateral motor neuron group of the contralateral spinal cord (in rats: Küchler et al., 2002; in cats: Holstege 1987b; McCurdy et al., 1987; Fujito and Aoki, 1995; in monkeys: Shapovalov et al., 1971; Ralston et al., 1988). Ipsilateral fibers are much fewer in number and they are similarly distributed in the gray matter as contralateral fibers (Antal et al., 1992; Holstege 1987b; Holstege and Tan, 1988). In the rat, these ipsilateral fibers are observed at all levels of the spinal cord (Antal et al., 1992), whereas in the cat they are only seen in cervical and upper
thoracic segments (Holstege 1987b; Holstege and Tan, 1988). The rubrospinal fiber termination has not been studied in the mouse.

1.2.2 The superior colliculus
It is involved in orientation to visual stimuli (in rats: Goodale et al., 1978; Sahibzada et al., 1986), influencing navigation and spatially guided movement (in rats: Cooper et al., 1998), the direction and speed of eye movements (in rats and hamsters: McHaffie and Stein 1982), and avoidance, defensive and escape reaction (in rats: Olds and Olds 1962; Redgrave et al., 1981; King 1999).

The deep layer of the superior colliculus (SC) issues projections to the contralateral spinal cord (in mice: VanderHorst and Ulfhake, 2006; in rats: Zemlan et al., 1979; Leong et al., 1984a; 1984b; in cats: Nyberg-Hansen, 1966; Kuypers and Maisky, 1975; Hayes and Rustioni, 1981; Olivier et al., 1991; Cowie and Holstege, 1992; Satoda et al., 2002) (anatomy of mouse SC shown in Fig1.5-7). Some studies also show the presence of spinal projecting neurons in the intermediate gray layer (in rats: Leong et al., 1984a; 1984b; Nudo and Masterton, 1989; in cats: Kuypers and Maisky, 1975; Hayes and Rustioni, 1981; Nudo and Masterton, 1989; Olivier et al., 1991; Cowie and Holstege, 1992; Satoda et al., 2002; in monkeys: Nudo and Masterton, 1989; May and Porter, 1992). These spinal projecting collicular neurons mainly concentrate in the posterior part of the superior colliculus (in cats: Olivier et al., 1991; in monkeys: Castiglioni et al., 1978). In the monkey, the arrangement of spinal projecting neurons is perpendicular to the surface of the superior colliculus, suggesting a columnar organization of spinal projecting neurons.

Fibers from the superior colliculus cross the midline at the level of the ventral tegmental decussation and dive into the ventral funiculus after the inferior olive. In the contralateral spinal cord, dense fibers are observed in the dorsolateral portion of lamina 7, with lesser dense fibers also seen in laminae 6, 8, and 9 of the upper cervical cord (in rats: Yasui et al., 1998; in cats: Nyberg-Hansen, 1966; Huerta and Harting, 1982; Cowie and Holstege, 1992). Ipsilateral fibers are also observed in the rat, but the density is less than that of the contralateral fibers (Yasui et al., 1998). The distribution of fibers from the superior colliculus in the mouse is still unknown.

1.2.3 The periaqueductal gray
Fig1.6 A coronal section at the level of the interpeduncular nucleus showing the structure of nuclei in the midbrain. The photograph on the left is a Nissl stained section, the diagram on the right is the corresponding drawing.


It contains a large number of spinal projecting neurons in all species studied (in mice: VanderHorst and Ulfhake, 2006; in rats: Basbaum and Fields, 1979; Mantyh and Peschanski, 1982; Leong et al., 1984a; Masson et al., 1991; Kudo et al., 1993; Li et al., 1993; in cats: Basbaum and Fields, 1979; Hayes and Rustioni, 1981; Mantyh and Peschanski, 1982; Cowie and Holstege, 1992; Mouton and Holstege, 1994; Satoda et al., 2002; in monkeys:
Castiglioni et al., 1978; Mantyh and Peschanski, 1982; Mantyh 1983; Carlton et al., 1985) (anatomy of mouse PAG shown in FigI.5-7). These neurons are predominantly located in the ipsilateral ventral and lateral parts of the PAG. A small number of neurons are present in the dorsal part (in rats: Leong et al., 1984a; Mantyh and Peschanski, 1982; in cats: Mantyh and Peschanski, 1982; Cowie and Holstege, 1992; Satoda et al., 2002; in monkeys: Mantyh and Peschanski, 1982).

In the monkey, descending fibers from the periaqueductal gray take a course in the hindbrain lateral to the medial longitudinal fasciculus (mlf) and follow in the lateral funiculus to the caudal lumbar cord. In the gray matter, fibers chiefly terminate in laminae 5 to 8 (Mantyh 1983). Mouton and Holstege (1994) reported that fibers from the cat periaqueductal gray travel in the ventral, ventromedial, and lateral funiculi and reach sacral levels. In the gray matter, these fibers terminate mainly in lamina 8, the medial part of lamina 7, and to a lesser extent in lamina 5. However, their injection site involved the adjacent cuneiform nucleus and this can not exclude the possibility that some fibers are from the cuneiform nucleus because the medial cuneiform nucleus also has spinal projections (Satoda et al., 2002).

1.2.4 The mesencephalic reticular formation
In primates and rodents, the mesencephalic reticular formation (mRt) is known for its role in saccadic eye movement (in rats: King and Fuchs 1979; in monkeys: Cohen and Buttner-Ennever 1984; Cohen et al., 1985; Waitzman et al., 1996; Waitzman et al., 2000a; 2000b; Waitzman et al., 2002; Cromer and Waitzman 2006). It is another major source of spinal projections in the midbrain. Spinal projecting neurons in this nucleus are adjacent to the interstitial nucleus of Cajal (Holstege and Cowie, 1989; Cowie and Holstege, 1992; Satoda et al., 2002) (anatomy of mouse mRt shown in FigI.6-7). Fibers from the mRt preferentially project to the ipsilateral spinal cord (in rats: Leong et al., 1984a; Auclair et al., 1993; de Boer-van Huizen and ten Donkelaar, 1999; in cats: Holstege and Cowie, 1989; Cowie and Holstege, 1992; Satoda et al., 2002; Warren et al., 2008; in monkeys: Castiglioni et al., 1978; Nudo and Masterton, 1988; Robinson et al., 1994). These fibers reach the lumbar segment in rats (Leong et al., 1984a) and cats (Holstege and Cowie, 1989). Spinal projections from this nucleus have not been reported in the mouse.

1.2.5 Edinger-Westphal nucleus
The Edinger-Westphal (EW) nucleus is not only involved in oculomotor adaptation (Westphal 1887; in cats: Burde et al., 1982; in humans: Horn and Adamczyk, 2012), but also
involved in stress (in mice: Weninger et al., 2000; in rats: Kozicz et al., 2001; Kozicz, 2003; Gaszner et al., 2004), anxiety (in rats: Skelton et al., 2000a; Skelton et al., 2004). It has spinal projecting neurons bilaterally with an ipsilateral predominance in the rat (Basbaum and Fields, 1979; Leong et al., 1984a; 1984b; Kudo et al., 1993), cat (Loewy and Saper, 1978; Basbaum and Fields, 1979; Holstege and Tan, 1988; Isa and Sasaki, 1992a), and monkey (Castiglioni et al., 1978; Carlton et al., 1985; Nudo and Masterton, 1988) (anatomy of mouse EW shown in FigI.6). Fibers from this nucleus travel in the dorsolateral funiculus of the monkey (Carlton et al., 1985) and terminate mainly in lamina 1, and in the cat also terminate to a lesser extent in lamina 5 (Loewy and Saper, 1978). Projections from this nucleus have not been studied in the mouse.

1.2.6 The dorsal raphe nucleus
The dorsal raphe nucleus (DR) is responsible for fear and stress response and modulating stress-related behaviours (in rats: Maier et al., 1993; Maier et al., 1994; Hammack et al., 2002). It is also involved in sleep (in rats: Portas et al., 1996; Monti et al., 2002; Manfridi et al., 2003; Lagos et al., 2009) and naloxone-induced morphine withdrawal (in rats: Alaei et al., 2002).

Studies show that this nucleus has spinal projections in a few species, but demonstrates different labeling patterns between species (anatomy of mouse DR shown in FigI.7). In the rat and monkey, a small number of neurons are present in this nucleus (Mantyh and Peschanski, 1982; Kazakov et al., 1993), but there are more neurons in the cat (Mantyh and Peschanski, 1982). Among these neurons projecting to the rat cervical cord, 90% are serotoninergic (Bowker et al., 1981). The proportion of serotoninergic spinal projecting neurons drops to 70% after lumbar cord injections of the retrograde tracer (Li et al., 1993). Fibers from this nucleus reach at least lumbar segments as reported so far in the rat, cat, and the monkey (Mantyh and Peschanski, 1982; Li et al., 1993). But in a study on the mouse, the dorsal raphe has not been reported to have spinal projections (VanderHorst and Ulfhake, 2006).

1.2.7 The cuneiform nucleus
The cuneiform nucleus (CnF) has been proposed to be part of the mesencephalic locomotion center (in cats: Garcia-Rill et al. 1983; in rats: Skinner and Garcia-Rill 1984; Coles et al. 1989; Allen et al. 1996). This nucleus contains spinal projecting neurons in the cat (Satoda et al., 2002) and monkey (Castiglioni et al., 1978) (the anatomy of mouse CnF shown in FigI.8).
In the cat, it has bilateral projections to the cervical cord with an ipsilateral predominance. Its neurons are larger than those in the adjacent periaqueductal gray (Satoda et al., 2002). In the monkey, spinal projecting neurons are ipsilaterally located in the medial part of this nucleus, similar to those in the cat. Descending fibers from this nucleus travel down to the cervical enlargement although the number of labeled fibers drops dramatically at lower cervical levels (Castiglioni et al., 1978). In the mouse and rat, this nucleus has not been reported to have spinal projections.

1.3. Rhombencephalon

1.3.1 The trigeminal nuclei
These nuclei are responsible for relaying sensory inputs from facial, oral, tooth pulp and pharyngolaryngeal tissues through their sensory subdivisions (in cats: Darian-Smith et al., 1968; Greenwood and Sessle 1976; review: Dubner and Bennett 1983) and innervating the chewing muscles, the tensor tympani, tensor veli palatini, mylohyoid, and anterior belly of the digastric through its motoneurons (in cats: Vedral and Matzke 1967; Keller et al., 1983;

![Fig1.7](image) A coronal section at the level of the pontine nuclei showing the structure of nuclei in the midbrain and rostral hindbrain. The photograph on the left is an AChE stained section, the diagram on the right is the corresponding drawing.

Yoshida et al., 1983; in rabbits: Matsuda et al., 1978; Passatore et al., 1983; in rats: Sasamoto 1979; Kemplay and Cavanagh 1983; in monkeys: Yassin and Leong 1979; Mizuno et al.,

The trigeminal nuclei encompass three subdivisions: the mesencephalic trigeminal nucleus (Me5), the principal sensory trigeminal nucleus (Pr5), and the spinal trigeminal nucleus (Sp5) (anatomy of mouse trigeminal nucleus shown in FigI.8-14).

The Me5 has a small number of spinal projecting neurons in its caudal part in the rat (Leong et al., 1984a; Kudo et al., 1993; Auclair et al., 1999), and these neurons are more clearly labeled in neonatal and juvenile animals (Leong et al., 1984b). In the rat and cat, fibers from these neurons only reach the upper cervical cord (in rats: Sirkin and Feng, 1987; in cats: Mizuno and Sauerland, 1970; Matsushita et al., 1981). Projections from the Me5 in mice have not been reported.

In rats, the Pr5 has a small number of spinal projecting neurons in its rostral part (Leong et al., 1984a; Diagne et al., 2006), but in cats, there are no labeled neurons after injections to the cervical and thoracic cord (Matsushita et al., 1981; Matsushita et al., 1982). In the mouse, projections from the Pr5 have not been reported.

In the spinal trigeminal nucleus, the oral part (Sp5O) has the largest number of spinal projecting neurons bilaterally (in rats: Ruggiero et al., 1981; Leong et al., 1984a; Nudo and Masterton, 1988; in cats: Matsushita et al., 1981; Nudo and Masterton, 1988; in monkeys: Nudo and Masterton, 1988). Interestingly, the rostral half of this nucleus has an ipsilateral predominance (spinal projecting neurons are mainly located in the dorsomedial portion of it) and the caudal half has a contralateral predominance as revealed by lumbosacral injections (Leong et al., 1984a). Ruggiero et al (1981) found that cervical projecting neurons in the rat Sp5O are bilaterally distributed, whereas thoracic and lumbosacral projecting neurons are contralaterally distributed. However, Diagne et al (2006) only found scattered cells in the rat Sp5O. The interpolar (Sp5I) and the caudal parts (Sp5C) of the spinal trigeminal nucleus have an ipsilateral predominance in the labeling of spinal projecting neurons and their fibers reach the lumbosacral segment (in rats: Leong et al., 1984a; Nudo and Masterton, 1988; Kudo et al., 1993; in cats: Matsushita et al., 1982; Nudo and Masterton, 1988; in monkeys: Nudo and Masterton, 1988). In the rat, spinal projecting neurons in the Sp5I are preferentially located in the ventral and lateral parts of this nucleus (Phelan and Falls, 1991). In contrast, in
Fig 1.8 A coronal section at the level of the rostral pole of locus coeruleus showing the structure of nuclei in the caudal midbrain and rostral hindbrain. The photograph on the left is an AChE stained section, the diagram on the right is the corresponding drawing.

In the cat, spinal projecting neurons are chiefly located in the ventromedial part of this nucleus (Matsushita et al., 1981). Spinal projecting neurons in the Sp5C are located in laminae 1, 3, 4, and 5 (in cats: Matsushita et al., 1982). Fibers from the Sp5C mainly terminate in the lateral cervical nucleus and the dorsal horn of upper cervical cord (in cats: Burton et al., 1979). In the mouse, spinal projections from this nucleus have not been investigated.

1.3.2 The vestibular nuclei
As a sensory entity, the vestibular nuclei receive primary afferents from the labyrinth of the inner ear. They also coordinate with visual and proprioceptive sensations to integrate information from the external environment, helping maintain the postural stability and gaze (in monkeys: Gdowski and McCrea 1999; 2000; Roy and Cullen 1998; 2001; in rats: Fuller 1985; Dieringer and Meier 1993; Lannou et al., 1982; Hess et al., 1989; Reber et al., 1996; Brettler et al., 2000; Plotnik et al., 1999; Niklasson et al., 1988; 1990).
The vestibular nuclei are four: the lateral (LVe), superior (SuVe), medial (MVe), and the spinal nuclei (SpVe) (anatomy of mouse vestibular nuclei shown in Fig. 9-12). Each of the subdivisions has been reported to have spinal projecting neurons and the largest is the lateral vestibular nucleus (in mice: Carretta et al., 2001; in rats: Watkins et al., 1981; Huisman et al., 1984; Nudo and Masterton, 1988; Shen et al., 1990; de Boer-van Huizen and ten Donkelaar, 1999; in cats: Kuypers and Maisky, 1975).

The LVe has spinal projecting neurons throughout the rostrocaudal extent after cervical injections of the retrograde tracer. The majority of them are large cells located in the dorsal half of the nucleus in the rat (Zemlan et al., 1979; Leong et al., 1984a), and both dorsal and ventral halves in the cat (Hayes and Rustioni, 1981). The spinal projecting neurons to cervical and lumbar enlargements are organized topographically (in cats: Nyberg-Hansen 1966; Hayes and Rustioni, 1981). Fibers from this nucleus travel bilaterally in the ventromedial fasciculus, ipsilaterally in the ventrolateral and lateral funiculi (in rats: Zemlan et al., 1979; in cats: Nyberg-Hansen, 1966; Rose et al., 1992; Rose et al., 1999). They reach the sacrocaudal or even the coccygeal segment (in rats: Masson et al., 1991; in cats: Hayes and Rustioni, 1981; Wada et al., 1993). Fibers from the LVe mainly terminate in the ventromedial gray matter (in rats: Huisman et al., 1984; Shen et al., 1990; in cats: Nyberg-Hansen, 1966; Rose et al., 1992; Rose et al., 1999). From E14.5, the LVe is retrogradely labeled in rat embryos (Auclaire et al., 1993; Kudo et al., 1993; de Boer-van Huizen and ten Donkelaar, 1999). In the mouse, retrogradely labeled neurons are exclusively found in the ipsilateral LVe (VanderHorst and Ulfhake, 2006), but the distribution of the vestibulospinal fibers in the spinal cord has not been investigated.

The MVe has a considerable number of spinal projecting neurons and most of them are located at the junction of the MVe and the SpVe (in mice: Carretta et al., 2001; in rats: Watkins et al., 1981; Leong et al., 1984a; in cats: Hayes and Rustioni, 1981; in monkeys: Kneisley et al., 1978). These neurons concentrate in the caudal half of this nucleus after lumbosacral injections, with more neurons in the rostral part of it after cervical injections of the retrograde tracer (in rats: Leong et al., 1984a; in cats: Hayes and Rustioni, 1981; in monkeys: Carlton et al., 1985). Some of these spinal projecting neurons are GABAergic in the rat (Stornetta and Guyenet, 1999; Valla et al., 2003). The MVe sends fibers bilaterally to the spinal cord with a contralateral predominance in rats (Huisman et al., 1984; Leong et al., 1984a; Valla et al., 2003), and an ipsilateral predominance in the cat (Nyberg-Hansen, 1966).

The spinal vestibular nucleus (SpVe) contains a large number of spinal projecting neurons (in mice: VanderHorst and Ulfhake, 2006; in rats: Nudo and Masterton, 1988; de Boer-van Huizen and ten Donkelaar, 1999; Valla et al., 2003; in cats: Nudo and Masterton, 1988; in monkeys: Carlton et al., 1985; Nudo and Masterton, 1988). The majority of labeled cells are seen in the caudal part of the nucleus (in rats: Zemlan et al., 1979; Leong et al., 1984a). Descending vestibulospinal fibers from this nucleus travel in the anterolateral column and terminate bilaterally in the spinal cord with a slight contralateral predominance (in mice: VanderHorst and Ulfhake, 2006; in rats: Zemlan et al., 1979; Watkins et al., 1981; Shen et al., 1990; in cats: Hayes and Rustioni, 1981). These vestibulospinal fibers reach the lumbar (in cats: Hayes and Rustioni, 1981) or sacrocaudal segments (in rats: Masson et al., 1991) and are distributed mainly in lamina 8 and 9 and, to a lesser extent in laminae 2-7 (Matesz et al., 2002). Immunohistochemical studies showed that some of the spinal projecting neurons are glutamic acid decarboxylase (GAD) reactive (in rats: Stornetta and Guyenet, 1999; Valla et al., 2003).

The SuVe has a few labeled neurons but most of them are located in the caudal part of the ipsilateral nucleus, separated by some fibers from the LVe (in rats: Leong et al., 1984a; Masson et al., 1991; Reed et al., 2008; in rats, cats, and monkeys: Nudo and Masterton, 1988). Descending vestibulospinal fibers from this nucleus reach the lumbar cord and terminate in the intermediate laminae (Reed et al., 2008). A small number of vestibulospinal fibers reaches sacrocaudal segments (Masson et al., 1991). In the mouse, the distribution of vestibulospinal fibers has not been studied.

1.3.3 The solitary nucleus
The solitary nucleus (Sol) has spinal projecting neurons in all species studied (in mice: Auclaire et al., 1999; VanderHorst 2005; VanderHorst and Ulfhake, 2006; in rats: Norgren 1978; Leong et al., 1984a; Mtui et al., 1993; in cats: Kuypers and Maisky, 1975; Loewy and
Burton, 1978; Basbaum and Fields, 1979; Rikard-Bell et al., 1984; in monkeys: Carlton et al., 1985) (anatomy of mouse Sol shown in Fig1.11-14). These neurons are distributed in the ventral (SolV), ventrolateral (SolVL), intermediate (SolI) and commissural (SolC) subdivisions of the contralateral nucleus (in mice: VanderHorst 2005; VanderHorst and Ulfhake, 2006; in rats: Leong et al., 1984a; in cats: Kuypers and Maisky, 1975; Loewy and Burton, 1978). However, some studies showed that these spinal projecting neurons are mainly located in the ipsilateral nucleus (in mice: Auclaire et al., 1999; in rats: Norgren 1978; Mtui et al., 1993; in monkeys: Carlton et al., 1985). Anterograde studies in different species also found different labeling patterns in the spinal cord. In the rat, an ipsilateral dominance is shown (in rats: Norgren 1978; Mtui et al., 1993). In contrast, in the cat, a contralateral predominance is reported (Loewy and Burton, 1978).

Leong et al (1984a) showed that both the rostral and caudal parts of this nucleus project to the cervical cord and only the caudal part projects to the lumbar cord. Descending fibers from this nucleus are intense in the upper cervical cord and the density drops dramatically in more caudal segments (in rats: Norgren 1978). These fibers travel in the lateral funiculus and give off collaterals to lamina 5, 7, and the intermediolateral cell column in thoracic segments (in rats: Mtui et al., 1993; in cats: Loewy and Burton, 1978). Masson et al (1991) showed that fibers from this nucleus reach the sacral cord in the rat. In the mouse, the termination of solitariospinal fibers has not been investigated. From E17.5 solitariospinal fibers begin to reach the spinal cord in rat embryos (Kudo et al., 1993).

### 1.3.4 Locus coeruleus and subcoeruleus nucleus

Locus coeruleus (LC) is involved in the generation of brain state including arousal (in rats: Aston-Jones and Bloom 1981), vigilance (in rats: Aston-Jones et al., 1991; in monkeys: Rajkowski et al., 1994), alerting, orienting, attention (in monkeys: Aston-Jones et al., 1999; in rats: Bouret and Sara 2004), also it is responsible for stress reaction (review: Valentino et al., 1993).

Locus coeruleus has bilateral projections to the spinal cord with an ipsilateral predominance (in mice: VanderHorst and Ulfhake, 2006; in rats: Guyenet 1980; Huisman et al., 1984; Leong et al., 1984a; Sluka and Westlund, 1992; in cats: Kuypers and Maisky, 1975; Hancock and Fougerousse, 1976; Basbaum and Fields, 1979; in monkeys: Hancock and Fougerousse, 1976; Kneisley et al., 1978; Carlton et al., 1985) (anatomy of mouse LC
shown in Fig. 9). Spinal projecting neurons are mainly located in the ventral portion of this nucleus (in mice: VanderHorst and Ulfhake, 2006; in rats: Zemlan et al., 1979; Guyenet 1980; Watkins et al., 1981; Huisman et al., 1984; Leong et al., 1984a; Kudo et al., 1993; in cats: Kuypers and Maisky, 1975). Some of these spinal projecting neurons are tyrosine hydroxylase reactive (in mice: VanderHorst and Ulfhake, 2006; in rats: Fritschy et al., 1987).

Fibers from the LC travel in the ventral funiculus and the ventral part of the lateral funiculus (in rats: Nygren and Olson, 1977; Zemlan et al., 1979; Fritschy et al., 1987; in monkeys: Carlton et al., 1985). Fibers reach the lumbosacral segment (in rats: Watkins et al., 1981; Leong et al., 1984a; Fritschy et al., 1987; Shen et al., 1990; Masson et al., 1991; in cats:

Fig. 9 A coronal section at the level of the superior olive showing the structure of locus coeruleus, Barrington’s, trigeminal, vestibular, raphe, and reticular nuclei in the hindbrain. The photograph on the left is a Nissl stained section, the diagram on the right is the corresponding drawing.

Kuypers and Maisky, 1975; Hancock and Fougerousse, 1976; Basbaum and Fields, 1979; in monkeys: Hancock and Fougerousse, 1976; Kneisley et al., 1978; Carlton et al., 1985). These fibers terminate preferentially in the dorsal horn of the spinal cord. Sparse fibers are also present in the intermediate zone and the motor neuron pool (in rats: Fritschy et al., 1987;
Fritschy and Grzanna, 1990; Clark and Proudfit, 1992). However, differences in terminating pattern among different strains also exist. In Sasco rats, coeruleospinal fibers are distributed in the medial part of laminae 7, 8, 9, and 10, whereas in Harlan rats, fibers mainly terminate in the dorsal horn (Clark and Proudfit, 1992; Sluka and Westulund, 1992). Furthermore, fibers from the LC decussate in the spinal cord (Commissiong 1981). This might explain why there is a controversy in the projecting pattern. The distribution of coeruleospinal fibers has not been studied in the mouse through anterograde tracing methods.

The subcoeruleus nucleus (SubC) is an important antinociceptive nucleus (in monkeys: Girardot et al., 1987; in rats: Tsuruoka et al., 2010; Tsuruoka et al., 2011). Unlike locus coeruleus, the subcoeruleus nucleus in the mouse has a different spinal projecting pattern, furnishing fibers mainly to the contralateral spinal cord (VanderHorst and Ulfhake, 2006) (anatomy of mouse SubC shown in Fig1.8). In contrast, in the rat, cat, and monkey, spinal projections mainly originate from the ipsilateral nucleus (rats: Guyenet 1980; Leong et al., 1984a; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979; in monkeys: Kneisley et al., 1978). In the rat, subcoeruleus fibers travel in the ventrolateral funiculus (Zemlan et al., 1979) or in the dorsolateral and ventral funiculi (Fritschy and Grzanna, 1990). In the cat, the fibers travel in the dorsolateral funiculus (Basbaum and Fields, 1979). These fibers predominantly terminate in the ventromedial gray matter in the lumbar cord in rats (Huisman et al., 1984; Fritschy and Grzanna, 1990; Shen et al., 1990). A5 is located ventral to the subcoeruleus nucleus and medial to the facial nerve. It has spinal projections bilaterally with a contralateral predominance. Its fibers reach the lumbar cord and terminate in deep laminae of the dorsal horn and the intermediate zone in the cervical cord. These fibers also terminate heavily in the intermediolateral cell column in the thoracic cord (anatomy of mouse spinal cord shown in Fig1.15). Fibers are sparse in the intermediate zone in the lumbar cord but are also present in the dorsal and ventral horns (in rats: Fritschy and Grzanna, 1990; Clark and Proudfit, 1993; Tavares et al., 1997).

**1.3.5 Dorsal column nuclei**
The cuneate (Cu) and gracile (Gr) nuclei have ipsilateral spinal projecting neurons in the rat (Leong et al., 1984a; Burton and Loewy 1977 and Nudo and Masterton, 1988 -including rats, cats, and monkeys; Shen et al., 1990; Kudo et al., 1993; Vallanueva et al., 1995), cat (Wada et al., 1993), and monkey (Burton and Loewy, 1977; Nudo and Masterton 1988) (anatomy of mouse Cu and Gr shown in Fig1.13 and 14). There are more spinal projecting neurons in the
cuneate nucleus than in the gracile nucleus, and the majority of these neurons are on the ventral border of these two nuclei (in rats: Leong et al., 1984a; Nudo and Masterton, 1988). But Zemlan et al (1979) demonstrated that most of the spinal projecting neurons are on the contralateral side. Fibers from the caudal part of the cuneate nucleus descend in the dorsal funiculus and terminate in the dorsal horn (mainly in lamina 4, 5, possibly in lamina 1) of the cervical cord (in rats: Villanueva et al., 1995). However, Burton and Loewy (1977) found that these fibers reach the lumbar cord in the rat, cat, and monkey. In the mouse, there are no similar reports.

1.3.6 Cerebellar nuclei
The cerebellum is playing an essential role in motor control by contributing to coordination, precision, and accurate timing (Ito 1984; Sultan and Their 2000). It is also involved in cognitive and affective function (in humans: Stoodley et al., 2011; Yeganeh-Doost et al., 2011). It has three deep nuclei: the lateral (Lat), interposed, and the medial (Med) cerebellar nuclei (anatomy of mouse cerebellar nuclei shown in Fig1.10 and 11). Among them,
interposed (especially in the anterior portion – IntA) and medial nuclei (Med) have been reported to have contralateral spinal projections (in rats: Leong et al., 1984a; 1984b; Nudo and Masterton, 1988-rats, cats, and monkeys; in monkeys: Batton et al., 1977; Asanuma et al., 1980). Fibers from the medial cerebellar nucleus terminate in the ventral horn at cervical level 1 (in monkeys: Batton et al., 1977), whereas fibers from the interposed cerebellar nucleus travel in the medial part of the ventral funiculus and terminate in the interneurons of the spinal cord (in monkeys: Asanuma et al., 1980). These cerebellar nuclei have not been studied in the mouse.

1.3.7 The ambigus nucleus and hindbrain respiratory nuclei

The ambiguus nucleus (Amb), solitary nucleus (Sol), the dorsal motor nucleus of vagus (10N), and the neighboring medullary reticular formation are involved in central control of swallowing (in humans: Martino et al., 2001; in cats: Ambalavanar et al., 2004). The ambiguus nucleus has been shown to have contralateral spinal projections in all species studied (in mice: VanderHorst and Ulfhake, 2006; Nudo and Masterton, 1988-including rats, cats, monkeys; in rats: Leong et al., 1984a; Hardy et al., 1998; Ellenberger 1999a; in cats: Lan et al., 1997) (anatomy of mouse counterparts shown in Fig.11-13). Spinal projecting neurons are distributed from the caudal pole of the Amb to the mid level of the inferior olive. Its fibers reach the lumbosacral segments in the rat (Leong et al., 1984a). In the gray matter of the spinal cord, fibers from the Amb terminate in the medial part of the ventral horn and the intermediolateral cell column in the rat (Hardy et al., 1998). Ventral to the Amb, some spinal projecting neurons are observed in the rostral ventral respiratory group and the Bötzinger complex (in rats: Ellenberger 1999).

Caudal to the Amb, the retroambiguus nucleus (RAmb) is located in the caudal hindbrain. It might be involved in respiration and vocalization (in monkeys: VanderHorst et al., 2000; in cats: Boer et al., 2006; Subramanian and Holstege 2009). It has been known for its bilateral spinal projections with a contralateral predominance (in mice: VanderHorst and Ulfhake, 2006; in rats: Leong et al., 1984a; 1984b; Holstege et al., 1997; Hardy et al., 1998; in hamster: Gerrits et al., 2000; Gerrits et al., 2004; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979) (anatomy of mouse RAmb shown in Fig.14). Its fibers travel in the lateral and ventrolateral funiculi and terminate in the ventral horn, some of which establish monosynaptic connections with the motor neurons innervating the cutaneous trunci, iliopsoas, lateral longissimus, quadratus lumborum, and abdominal external oblique muscle (in hamster:
Gerrits et al., 2000; Gerrits et al., 2004; in cats: Boers et al., 2006). The fiber terminals of the ambiguus and the retroambiguous nuclei in the mouse spinal cord have not been investigated.

1.3.8 Raphe magnus, raphe obscurus, raphe pallidus nuclei
The raphe magnus nucleus (RMg) is involved in pain perception and it usually has antinociceptive influence (in cats: Fields et al., 1977; in rats: Hammond et al., 1980; Brodie and Proudfoot 1986; Dickenson and Goldsmith 1986; Llewelyn et al., 1986; Inase et al., 1987; Clatworthy et al., 1988; Hentall and Fields 1988; in rabbits: Sotgiu 1987). The raphe obscurus (ROb) and pallidus (RPa) nuclei have also been suggested to be involved in nociceptive response (in cats: Conrath-Verrier et al., 1983; in rats: Dantas et al., 1990; in mice: Sluka et al., 2011).

Rhombocephalic raphe nuclei are known to be the major source of serotonergic projections to

**Fig 1.11** A coronal section at the level of the rostral ambiguous nucleus showing the structure of the ambiguus, trigeminal, vestibular, cerebellar, raphe, and reticular nuclei in the hindbrain. The photograph on the left is an AChE stained section, the diagram on the right is the corresponding drawing.
the spinal cord (in mice: VanderHorst and Ulfhake, 2006; Braz and Basbaum, 2008; in rats: Bowker et al., 1981; in monkeys: Bowker et al., 1982). These nuclei are located along the midline in the hindbrain, however, they have columns of neurons off the midline (anatomy of mouse raphe nuclei shown in Fig1.8-12). Spinal projections originate from the ipsilateral raphe nuclei (in mice: Carretta et al., 2001; Vander Horst and Ulfhake, 2006; Braz and Basbaum, 2008; in rats: Leichnetz et al., 1978; Zemlan et al., 1979; Bowker et al., 1981; Leong et al., 1984a; Masson et al., 1991; Allen and Cechetto, 1994; in cats: Kuypers and Maisky, 1975; Hayes and Rustioni, 1981; Kausz 1991; Wada et al., 1993; in monkeys: Kneisley et al., 1978; Carlton et al., 1985). The majority of these spinal projecting neurons are serotonergic (in mice: Vander Horst and Ulfhake, 2006; Braz and Basbaum, 2008; in rats: Bowker et al., 1981; Bowker et al., 1983; Leanza et al., 1995).

Fibers from these raphe nuclei reach all levels of the spinal cord (in rats: Bowker et al., 1981; Watkins et al., 1981; Allen and Cechetto, 1994; in cats: Wada et al., 1993). Fibers from the RMg travel in the lateral funiculus and/or ventral funiculus (in rats: Zemlan et al., 1979; Watkins et al., 1981; in cats: Bobillier et al., 1976; Basbaum and Fields, 1979; in monkeys: Carlton et al., 1985) and terminate on forelimb motor neurons in the cervical cord (in cats: Alstermark et al., 1987), and in the intermediolateral cell column (anatomy of mouse spinal cord shown in Fig1.15) and respiratory neurons in the thoracic cord (in rats: Gilbey et al., 1995). In the lumbar cord, fibers from the RMg and RPa terminate in all laminae with a majority terminating in laminae 5, 6, and the ventral horn (in rats: Jones and Light, 1990). In the mouse, the distribution of raphespinal fibers has not been studied.

1.3.9 The paralemniscal nucleus
The paralemniscal nucleus (PL) is also called the paralemniscal reticular nucleus in some species (in rats: Leichnetz et al., 1978; Watkins et al., 1981; in cats: Basbaum and Fields, 1979; in monkeys: Carlton et al., 1985) (anatomy of mouse PL shown in Fig1.7). In physiological studies, this nucleus has been implicated to be involved in audio-vocalization (in bats: Suga and Schlegel, 1972; Suga and Shimozawa, 1974; Metzner, 1989; 1996; in monkeys: Kirzinger and Jürgens, 1991). It is also involved in nociceptive response (in rats: Hardy et al., 1983). This nucleus has contralateral spinal projections in the species studied (in rats: Watkins et al., 1981; Leong et al., 1984a; Nudo and Masterton, 1988; Reiner et al., 2008; in cats: Basbaum and Fields, 1979; in monkeys: Kneisley et al., 1978; Carlton et al., 1985). Fibers from this nucleus descend in the dorsolateral funiculus (in cats: Basbaum and Fields,
1979; in monkeys: Carlton et al., 1985) and can reach the lumbosacral segments (in rats: Leong et al., 1984a; Reiner et al., 2008; in monkeys: Carlton et al., 1985). These fibers might terminate mainly in the dorsal horn of the spinal cord and to a lesser extent in the ventral horn (in rats: Reiner et al., 2008).

1.3.10 The Barrington’s nucleus
The Barrington’s nucleus (Bar) is the micturition center (in rats: Matsuura et al., 2000; in cats: Sasaki 2002; 2005; in guinea pigs: Kuipers et al., 2007) and also involved in distal colon function (in rats: Pavcovich et al., 1988; Rouzade-Dominguez et al., 2003). It has bilateral spinal projections with an ipsilateral predominance (anatomy of mouse Bar shown in FigI.9). Its fibers can reach the lumbosacral cord (in mice: VanderHorst and Ulfhake, 2006; in rats: Russo et al., 2004; in guinea pig: Kuipers et al., 2007) and terminate mainly in the intermediolateral and intermediomedial cell columns (anatomy of mouse spinal cord shown in FigI.15), to a lesser extent in the ventral horn (in guinea pig: Kuipers et al., 2007). In the mouse, fiber termination of this nucleus in the spinal cord has not been studied.

1.3.11 The parabrachial and Köllike-Fuse nuclei
These two nuclei belong to the rostral pontine respiratory neuronal group and function as a pneumotaxic center promoting inspiration phase to terminate (in cats: St. John et al., 1971; Gautier and Bertrand, 1975; Caille et al., 1981; Dick et al., 1994). However, the lateral parabrachial nucleus also mediates shortening of expiration and increase of inspiratory drive during hypercapnia and hypoxia (in rats: Song and Poon 2009a; 2009b).

These two nuclei have bilateral spinal projections with an ipsilateral predominance (in rats: Leong et al., 1984a; in cats: Hayes and Rustioni, 1981; in monkeys: Carlton et al., 1985; Nudo and Masterton, 1988) (anatomy of mouse PB and KF shown in FigI.8). Most of these spinal projecting neurons are in the ventral part of the parabrachial nucleus (in rats: Leichnetz et al., 1978; in cats: Basbaum and Fields, 1979; Hayes and Rustioni, 1981). However, Basbaum and Fields (1979) reported that spinal projecting neurons are present in both the medial and the ventrolateral parts of the parabrachial nucleus in the cat. Westlund and Coulter (1980) showed that spinal projecting neurons in the monkey are located in the medial part of the parabrachial nucleus. Carlton et al (1985) found that spinal projecting neurons in the monkey are located in the dorsal part of the parabrachial nucleus instead of the ventral part. Nudo and Masterton (1988) claimed that spinal projecting neurons are scattered in the
A coronal section at the level of the rostral hypoglossal nucleus showing the structure of trigeminal, solitary, vestibular, and reticular nuclei in the hindbrain. The photograph on the left is an AChE stained section, the diagram on the right is the corresponding drawing.

parabrachial nucleus in rats, cats, and monkeys. Descending fibers from the parabrachial and Köllike-Fuse nuclei follow in the dorsolateral funiculus and reach the lumbosacral cord (in rats: Leong et al., 1984a; in cats: Basbaum and Fields, 1979; Hayes and Rustioni, 1981; in monkeys: Carlton et al., 1985). These fibers are distributed mainly in the dorsal horn, and to a lesser extent in the region surrounding the central canal and the ventral horn (in monkeys: Westlund and Coulter, 1980).

### 1.3.12 The reticular nuclei in the hindbrain
The reticular formation in the hindbrain is not only essential for wakefulness and arousal (Moruzzi and Magoun 1949; Bonvallet and Bloch 1961; in cats: Hobson et al., 1974; Xi et al., 1999; in rats: Camacho-Arroyo et al., 1991; Sanford et al., 2003), it is also important in motor control, nociceptive response.

The pontine reticular nucleus has a considerable number of spinal projecting neurons in its oral (PnO) and caudal (PnC) subdivisions (in mice: Carretta et al., 2001; VanderHorst and
Fig. 13 A coronal section at the level of inferior olive showing the structure of trigeminal, solitary, dorsal column, and reticular nuclei in the hindbrain. The photograph on the left is an AChE stained section, the diagram on the right is the corresponding drawing.

Ulfhake, 2006; in rats: Zemlan et al., 1979; Leong et al., 1984a; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979; Hayes and Rustioni, 1981; in monkeys: Carlton et al., 1985) (anatomy of mouse PnO and PnC shown in Fig. 7-9). The oral pontine reticular nucleus is responsible for wakefulness and anaesthesia (in rats: Camacho-Arroyo et al., 1991; Sanford et al., 2003; in cats: Xi et al., 1999).

Most spinal projecting neurons are on the ipsilateral side (in mice: Carretta et al., 2001; VanderHorst and Ulfhake, 2006). However, in the oral pontine reticular nucleus, there are more spinal projecting neurons in the rostral part of the contralateral nucleus than in the ipsilateral nucleus (in rats: Leong et al., 1984a; in cats: Kuypers and Maisky, 1975). Fibers from the caudal pontine reticular nucleus travel in the ventral funiculus and reach the sacral cord. In the gray matter of the spinal cord, they terminate in laminae 7, 8, and 9 (in rats:
Fig.1.4 A coronal section at the level of the pyramidal decussation showing the structure of trigeminal, solitary, dorsal column, and reticular nuclei in the hindbrain. The photograph on the right is a Nissl stained section, the diagram on the left is the corresponding drawing.

Sirkin and Feng, 1987; in cats: Nyberg-Hansen, 1965). Though fibers from the oral pontine reticular nucleus are not seen after lumbar or lower level spinal injections of the retrograde tracer (in rats: Sirkin and Feng, 1987; Masson et al., 1991), their terminal distribution in the spinal cord is similar to that of the caudal pontine reticular nucleus in the cervical and thoracic segments (in rats: Sirkin and Feng, 1987; in cats: Nyberg-Hansen, 1965). At E14.5, reticulospinal fibers from the pontine reticular nucleus have already reached the thoracic cord (Kudo et al., 1993).

The gigantocellular reticular nucleus (Gi) has an inhibitory influence on the cardiovascular response (in cats: Kuo et al., 1979; Hwa and Chan 1981; Chan and Chan 1983; Su et al., 1991; in rats: Aicher and Reis 1997; Aicher and Drake 1999).

It has a large number of spinal projecting neurons with an ipsilateral predominance (in mice: VanderHorst and Ulfhake, 2006; in rats: Watkins et al., 1981; Leong et al., 1984a; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979; Hayes and Rustioni, 1981; Wada et al., 1993) (anatomy of mouse Gi shown in Fig.10-12). Interestingly, the dorsolateral part of
this nucleus has more spinal projecting neurons on the contralateral side than on the ipsilateral side (in rats: Leong et al., 1984a). Both the ventral part of this nucleus including the alpha (GiA), ventral subdivisions (GiV), and the dorsal part of the gigantocellular reticular nucleus have spinal projecting neurons. Among these subdivisions, the GiV has the largest number of spinal projecting neurons (in mice: VanderHorst and Ulfhake, 2006; in rats: Zemlan et al., 1979; Shen et al., 1990). Lateral to the Gi, the lateral paragigantocellular reticular nucleus (LPGi) also has spinal projections predominantly from the ipsilateral side (in mice: VanderHorst and Ulfhake, 2006; in rats: Leong et al., 1984a; in cats: Kausz 1991; in monkeys: Carlton et al., 1985). Fibers from the Gi follow mainly in the dorsolateral funiculus, and to a lesser extent in the lateral and ventral funiculi. These fibers terminate in the ventral horn (some terminate on motor neurons), intermediate zone, the area surrounding the central canal, deep layer of the dorsal horn and in the lamina 1 of the lumbar cord (in rats: Holstege 1991; Hermann et al., 2003). However, the dorsolateral part of the gigantocellular reticular nucleus

**Fig1.15** A coronal section of the 6th thoracic cord showing the structure of the mouse spinal cord [from Atlas of the mouse spinal cord. In: Watson C, Paxinos G, Kayalioglu G (eds) The spinal cord. 2009, 1st edn. Elsevier Academic Press, San Diego]. The photograph on the left is a Nissl stained section, the diagram on the right is the corresponding drawing.
only terminates in thoracic segments (in cats: Kausz 1991). The lateral part of the
gigantocellular reticular nucleus has monosynaptic contact with preganglionic neurons in the
thoracic cord (Aicher et al., 1995). The dorsal (MdD) and ventral (MdV) medullary reticular
nuclei have bilateral spinal projections (in mice: VanderHorst and Ulfhake, 2006; in rats:
Leichnetz et al., 1978; Watkins et al., 1981; Leong et al., 1984a; Reed et al., 2008; in cats:
Basbaum and Fields, 1979; Hayes and Rustioni, 1981; in monkeys: Kneisley et al., 1978;
Carlton et al., 1985) (anatomy of mouse MdD and MdV shown in Fig.13 and 14). Some of
the spinal projecting neurons are glutamic acid decarboxylase (GAD) positive (in rats:
Ellenberger 1999). Fibers from the rat MdD terminate in the superficial layers of the dorsal
horn (Almeida et al., 2000). It is regarded as a prenociceptive center which facilitates pain
perception (Lima and Almeida 2002). The reticulospinal fibers from the MdV mainly travel
in the ventral funiculus (in cats: Nyberg-Hansen, 1965; Basbaum and Fields, 1979) and reach
the lumbosacral segment (in rats: Leong et al., 1984a; in monkeys: Kneisley et al., 1978;
Carlton et al., 1985). These fibers terminate in lamina 7 and to a lesser extent in adjacent

Between the MdD and MdV, the intermediate reticular nucleus (IRt) has a small number of
spinal projecting neurons and some of them are also GAD positive (in rats: Ellenberger 1999).
From E13 of the rat embryo, reticulospinal fibers from the medulla have reached the C3
segment (Auclair et al., 1993).

In addition to above reticular nuclei, the parvicellular reticular nucleus (PCRt) also has spinal
projecting neurons (in rats, cats, and monkeys-Nudo and Masterton, 1988) (anatomy of
mouse PCRt shown in Fig.9-12). This nucleus has premotor neurons of chewing muscles (in
rats: Mogoseanu et al., 1993; Satoh et al., 2006; Van Daele et al., 2011). Fibers from this
nucleus only reach the upper cervical cord. In the mouse, the fiber terminals from these
reticular nuclei have not been studied.

In summary, a large number of neuronal groups has been reported to the spinal cord in
various species. However, the spinal projecting neuronal groups in the mouse have not been
comprehensively studied. Furthermore, the mouse has been increasingly used in genetic and
spinal cord injury research. Therefore, this thesis aims to map all the spinal projecting
neuronal groups in the mouse and investigate the spinal cord terminations of some of these
nuclei.
CHAPTER II. Projections from the brain to the spinal cord in the mouse
Introduction

Pathways from the brain to the spinal cord in mammals play an important role in the initiation of movements of the limbs and trunk, including grasping, locomotion, respiration, and posture maintenance. The origin of these pathways has been studied in a wide variety of mammals. The best known centers that give origin to these pathways are the cerebral cortex (Miller 1987; Nudo and Masterton 1988; 1990; Galea and Darian-Smith 1994), the red nucleus (Pompeiano and Brodal 1957; Nyberg-Hansen and Brodal 1963; Carlton et al. 1985; Xu and Martin 1989; Kuchler et al. 2002), the brainstem reticular formation (Torvik and Brodal 1957; Nyberg-Hansen 1965; Peterson et al. 1975; Basbaum and Fields 1979; Nordlander et al. 1985; Sirkin and Feng 1987; Cruce et al. 1999; Satoda et al. 2002), and the vestibular nuclei (Peterson and Coulter 1977; Hayes and Rustioni 1981; Kimmel et al. 1982; Leong et al. 1984; Nudo and Masterton 1988; Masson et al. 1991; Wada et al. 1993; Lakke 1997). Apart from these well known centers of origin of the descending tracts, there are a large number of other nuclei that are labeled in retrograde tracing studies following spinal cord injections. These include the paraventricular nucleus (Berk and Finkelstein 1983; Okado and Oppenheim 1985; Kunzle 1992), the medial and interposed cerebellar nuclei (Med, IntP) (Bangma et al. 1984; Leong et al. 1984; Gross and Oppenheim 1985; Nudo and Masterton 1988; Sanchez-Camacho et al. 2001a), and the spinal trigeminal nucleus (Burton and Loewy 1977; Leong et al. 1984; Diagne et al. 2006). The relative size of these spinally projecting cell groups varies between species (Nudo and Masterton 1988).

There have been a few studies on the centers that give rise to the descending pathways in the mouse (Sbriccoli et al. 1995; Carretta et al. 2001; Tsukamoto et al. 2003). However, none of these studies attempted comprehensive description of the centers which give rise to the descending pathways. We believe there is a need for comprehensive baseline study which maps the cells of origin of these descending pathways to the spinal cord in the mouse. This will help other studies on the assessment of recovery after experimental spinal cord injuries. We have therefore attempted to reveal the spinal projecting neuron groups in the mouse by injecting retrograde tracers HRP and FG into the upper cervical spinal segments in a large series of mice.

Materials and Methods
Animals
36 C57/BL6 mice (10-12 weeks old, weight 25-30g) were used for this study. Mice were obtained from the Animal Resource Center of Western Australia. Large injections of retrograde tracer were made into upper cervical cord segments. The procedure was approved by the Animal Care and Ethics Committee of the University of New South Wales (07/108B, 08/48B).

Injection of retrograde tracer
Mice were anaesthetized with an intraperitoneal injection of ketamine (67mg/kg) and xylazine (10mg/kg). They were then mounted in a mouse stereotaxic head holder (Kopf Instruments). A 5 µl Hamilton syringe was mounted on a micromanipulator for spinal cord injection. The mouse adaptor was adjusted for optimal exposure of the upper cervical vertebrae. The first and second cervical spinal cord segments were exposed by C2 laminectomy. The dura on the right side was incised with the tip of a 29G needle and the 5µl Hamilton syringe was driven through this opening. The total amount of the retrograde tracer is 20-40 nl. For multiple injections, 2 or 3 injections were made at similar level in order to cover the mediolateral dimension of the spinal cord with a total amount of 20-40 nl. The syringe was left in place for 10 minutes following the injection. Fluoro-Gold (Fluorochrome, Denver, Co, USA) was diluted to 5% with distilled water and horseradish peroxidase (HRP, Sigma, type VI) was diluted to 30% with distilled water. In 12 cases HRP was injected, and in 18 cases FG was used. A control group of mice received normal saline injections into the spinal cord (3 cases each) and tracer injections into the cistern magna (3 cases each). The soft tissue and the skin were sutured and tetracycline was sprayed over the incision. Temgesic (buprenorphine) was injected subcutaneously to relieve pain for two days after surgery.

Tissue preparation
After a period ranging from 48 to 96 hours, mice were anesthetized with a lethal dose of pentobarbitone sodium (0.1 ml, 200mg/ml) intraperitoneally and perfused through the left ventricle, first with 60 ml of 0.9% normal saline with heparin (Sigma, 150IU/mouse), followed by 80 ml of 4% paraformaldehyde (Sigma) in 0.1 M phosphate buffer (PB, pH 7.4), and then 80 ml of 10% sucrose. The brain and cervical spinal cord were removed and postfixed in 4% paraformaldehyde for two hours at 4°C, followed by cryoprotection in 30% sucrose in 0.1 M PB solution (pH 7.4) overnight at 4°C. Serial sagittal or coronal sections of the whole brain were cut at 40µm thickness using a cryostat. Coronal sections (40µm) of the C1-C4 spinal cord segments were also cut using the cryostat. In cases of HRP injection,
sections were directly mounted onto gelatinized slides and dried for more than 2 hours at room temperature, then stained by 3,3′,5,5′-tetramethylbenzidine (TMB) (Mesulam 1978). In the case of FG, sections were mounted onto gelatinized slides, coverslipped with the anti-fade fluorescent mounting medium (Dako). HRP and Nissl sections were photographed with a Spot Insight camera mounted on an Olympus Provis microscope. FG labeled sections were examined with an Olympus fluorescence microscope equipped with a Zeiss Axio camera.

**Mapping**

Labeled neurons of all sections were analysed and mapped onto templates taken from the mouse brain atlas of Franklin and Paxinos (2008). The names and abbreviations of neuronal groups are those of Paxinos and Watson (2007). The mapping of labeled cells was assisted by reference to adjacent Nissl stained sections.

**Cell counting**

The labeled neurons were counted in two cases. The cases chosen were those that seemed to have the greatest number of labeled neurons in most areas. Labeled neurons were counted in every seventh section. The cells were counted with a Nikon Eclipse 80i microscope attached to an Optronics camera (Goleta, CA) which was in turn connected to a Dell Precision T3500 workstation using Stereoinvestigator software (MicroBrightfield, Williston, VT). The major boundaries of the section were drawn at 2× magnification, and labeled neurons were identified at 10× magnification. Each labeled cell was marked by a dot on the drawing. Labeled cells were identified on the basis that they contained a nucleus. In many cases a nucleolus was seen, but no attempt was made to ensure that each counted cell contained a nucleolus. For this reason, the counts should be considered to be general estimates of labelled neuron numbers. They are obviously not definitive counts.

**Results**

Both HRP and FG injections labeled cells in a wide range of brain regions. For the FG injections, different survival times were tried, but there was no significant increase in the number of labeled cells in the motor cortex after 96 hours survival. We therefore chose 96 hours as the survival time for the majority of the FG experiments. For the HRP injections, the survival time was 48 hours. While both HRP and FG techniques labeled cells in the same areas, the intensity of signal was greater with FG injections. For this reason, we have principally used the FG data for the analysis reported here. A typical injection site is shown in the lower right corner of FigII.1. The estimates of the number of labeled cells in each
region are listed in TableII.1. The control mice with tracer injections into the cistern magna did not have labelled neurons in the brain.

**FigII.1** In this diagram of a coronal section through the caudal hindbrain, at the level of the rostral pole of the thalamus, labeled neurons are shown in the motor and somatosensory cortical areas. The density of labelled neurons was greater on the contralateral side. Labelled neurons were not present in S1BF and S1ULp. The density of labeling in M1 and M2 was higher than that in S1 (S1HL, S1FL) and S2 (S2 cells were not labeled at this level). A line of labeled neurons was present in STMPL. [In Figures 1-15 labeled cells have been plotted on the diagrams of coronal sections from the mouse brain atlas of Franklin and Paxinos (2008). The interaural (anteroposterior) coordinate is shown on the bottom left]. A photomicrograph of a typical injection site at C3/4 level is shown in the bottom right hand corner (its scale bar is 200 micrometers). The photomicrograph in the upper left shows labeled cells in layer 5 of S1FL. The photomicrograph in the upper right shows labeled cells in layers 5 and 6 of M1 and M2. In these and all subsequent photomicrographs, the scale bar represents 50 micrometers.

**Cerebral cortex**
Labeled neurons were found in the primary and secondary motor cortex (M1, M2) (**FigII.1-2, 16-18**), and in the limb area (S1FL, S1HL), trunk area (S1Tr), shoulder area (S1Sh), and the dysgranular zone (S1DZ) of the primary somatosensory cortex (S1) on the contralateral side. Labeled neurons were also seen in the secondary somatosensory cortex (S2) on the
contralateral side (FigI.1-3, 18). The barrel field (S1BF) and upper lip (S1ULp) regions of
the primary somatosensory cortex contained no labeled cells. The labeled cortical neurons
were almost all pyramidal neurons in layer 5 and 6 [reference to the Chemoarchitectonic atlases
of the rat brain (2009)] with tear-shaped cell soma and long dendrites extending towards the
pial surface. Labeled neurons were more plentiful in the motor cortex than in the
somatosensory cortex. In M1, M2, and S1, the labeled neurons were arranged in three or
more rows within layer 5. In S2, only one or two layers of labeled neurons were observed. A
small number of labeled neurons were present in the same motor and somatosensory cortical
areas of the ipsilateral side.

![Diagram](image)

**FigI.2** In this diagram of a coronal section through the caudal hindbrain at the level of the
caudal paraventricular nucleus, labeled neurons are shown in M1, M2, S1 (S1HL, S1Sh,
S1DZ,), and S2, with a contralateral predominance. Large numbers of neurons were labeled
in the paraventricular hypothalamic nucleus, mainly in PaPo. Some neurons were labeled in
PLH, TuLH, RChL, and VMH and a few neurons were labeled in CeM. All the hypothalamic
and amygdaloid labeling was on the ipsilateral side except for PLH which has bilateral
labeling. The photomicrograph on the left shows labeled cells in the PaMP and PaPo. The
photomicrograph on the right shows a few labeled cells in the CeM nucleus of the amygdala.

**Amygdala and bed nucleus of stria terminalis**
In two cases, a small number of lightly labeled neurons were seen in the central part of the
extended amygdala (EAC), the medial division of the central amygdoid nucleus (CeM), and the anterior basolateral amygdaloid nucleus (BLA) on the ipsilateral side (FigII.2-3, 18). In one case, a few labeled neurons were found in the posterolateral part of the medial division of the bed nucleus of stria terminalis (STMPL). These neurons were in a line parallel to fibers of the internal capsule (ic) (FigII.1).

**Hypothalamus**
Labeled neurons were present in the rostral anterior parvicellular part (PaAP) and the caudal posterior part (PaPo) of the paraventricular hypothalamic nucleus. A prominent cluster of densely labeled cells was found in the caudal posterior part of the paraventricular hypothalamic nucleus, where they formed a compact hook shaped graph adjacent to the medial part of ZID (FigII.2 and 16). Ventral to PaPo, the ventromedial hypothalamic nucleus (VMH) and the lateral part of the retrochiasmatic nucleus (RChL) contained a few labeled cells. Labeled cells were also found in the peduncular part of the lateral hypothalamus (PLH), magnocellular nucleus of the lateral hypothalamus (MCLH), and the adjacent tuberal region of lateral hypothalamus (TuLH) (FigII.2-4, 16-17). There were a few labeled neurons in the subthalamic nucleus (STh) (FigII.3). Note that the subthalamic nucleus should be considered to be part of the hypothalamus from a developmental standpoint (Puelles et al. 2007). The posterior hypothalamic nucleus (PH) contained a few lightly labeled cells (FigII.4). The labeled hypothalamic cells were almost all ipsilateral, except those in PLH, which was bilaterally labeled with an ipsilateral predominance.

**Diencephalic nuclei**
At the coronal level of dorsomedial hypothalamic nucleus (DM), the caudal part of the paraxiphoid nucleus (PaXi) contained one or two labeled neurons in each section (FigII.3). A few cells in the subparafascicular thalamic nucleus (SPF) (FigII.4) were labeled, but they were not as strongly labeled as those in the nucleus of dorsal zona incerta (ZID) (FigII.3-4, 18) at the same level. Some labeled neurons were identified in H field of Forel (H) (FigII.4, 16-17) and there were labeled neurons present in the caudal part of the parafascicular nucleus (PF) of the ipsilateral side (FigII.4 and 16). More caudally, a dense cluster of neurons was found in an area which seemed to be either the lithoid nucleus (Lth) or the periaqueductal gray (PAG). We were unable to distinguish a clear boundary between these structures.
Labeled neurons were also found in the adjacent retroparafascicular nucleus (RPF) (FigII.16). These diencephalic nuclei were mainly labeled ipsilaterally. More caudally, the nucleus of Darkschewitsch (Dk) and interstitial nucleus of Cajal (InC) contained a few labeled cells with
an ipsilateral dominance (FigII.5 and 6). In regions at the rostral level of the posterior commissure (pc), two or three neurons were seen in the nucleus of posterior commissure (PCom) on both sides with a contralateral predominance (FigII.16). The magnocellular nucleus of posterior commissure (MCPC) also contained a few labeled neurons on the contralateral side (FigII.5 and 16).

FigII.3 In this diagram of a coronal section through the caudal hindbrain at the level of STh, labeled neurons are shown in contralateral S1Tr and S2. In the hypothalamus, labeled neurons were present ipsilaterally in PLH, MCLH, VMH, and STh. In the prethalamic region, labeled neurons were present in the PaXi and a few labeled neurons were seen in the ipsilateral BLA. The photomicrograph in the upper left shows labeled cells in layer 5 of the trunk region of S1 (S1Tr).

Red nucleus
Many large neurons were strongly labeled in the magnocellular part of the red nucleus (RMC) of the contralateral side, with fewer positive neurons in the parvicellular part of the red nucleus (RPC) on both sides (FigII.5-6, 16-17). Labeled neurons of RMC were evenly distributed from medial to lateral. In sagittal sections, more neurons were found in the caudal part than in the rostral part (FigII.16 and 17).
Mesencephalic reticular formation and tectal nuclei
A few labeled neurons were seen in the mesencephalic reticular nucleus (mRt) (FigII.6-8, 17-18). These cells were spread over a large area of the midbrain, but were sparsely distributed on the contralateral side. Labeled neurons on the ipsilateral side were more densely packed than the contralateral side at the level of the caudal pole of the medial geniculate (MG) and more caudally. The ipsilateral lateral periaqueductal gray (LPAG) contained a small number of labeled neurons. More caudally, neurons of the dorsomedial periaqueductal gray (DMPAG) were labeled as well. LPAG and ventrolateral periaqueductal gray (VLPAG) were both labeled on the ipsilateral side in coronal sections at the level of the oculomotor nucleus (3N) (FigII.6-8). In sagittal sections, the labeled neurons in PAG formed a column extending from ventral to the inferior colliculus (IC) to prosomere 1, bending rostroventrally at the junction of the superior colliculus (SC) and the pretectum (FigII.16). These labeled neurons were small and spindle shaped. Ventral to VLPAG, the cells of the supraoculomotor

FigII.4 In this diagram of a coronal section through the caudal hindbrain at the level of the dorsal lateral geniculate nucleus (DLG), labeled neurons are shown in bilateral LH and ipsilateral PH. In the diencephalon, labeled neurons were present in the medial part of PF, SPF and A11 cell group. Labeled neurons were also seen in the ipsilateral H and ZID. The photomicrograph in the upper right shows labeled cells in PF. The photomicrograph in the middle right shows labeled cells in ZID. The photomicrograph in the lower right shows labeled cells in SPF and the adjacent A11 cell group.
periaqueductal gray (Su3) were labeled bilaterally with an ipsilateral dominance (FigII.6 and 7). More ventrally, cells of the pre-Edinger-Westphal (PrEW), Edinger-Westphal (EW), and the medial accessory oculomotor (MA3) nuclei were lightly labeled bilaterally with an ipsilateral predominance.

In the caudal SC, there were two or three labeled neurons in the deep white (DpWh) and deep gray (DpG) layers in each section on the contralateral side (FigII.6). Laterally and caudally, there were five to ten neurons labeled in the region through the rostral 1/3 of the precuneiform area (PrCnF) on the ipsilateral side (FigII.7-8, 17).

FigII.5 In this diagram of a coronal section through the caudal hindbrain at the level of the medial geniculate nucleus (MG), labeled neurons are shown in InC and Dk on both sides. Labeled neurons were present in the ipsilateral PAG and contralateral MCPC. A few labeled neurons were found in MA3 (ipsilaterally), PrEW (in the midline), and RPC (bilaterally). The photomicrograph in the upper left shows labeled cells in MCPC.

Vestibular nuclei
Labeled neurons were found in all major nuclei of the vestibular complex. The lateral (LVe) and the superior (SuVe) vestibular nuclei were ipsilaterally labeled (FigII.10 and 11). The medial vestibular nucleus (MVe) and the spinal vestibular nucleus (SpVe) were bilaterally labeled (FigII.10-13, 16-18). The labeled neurons were mainly large stellate cells and the
labelling was intense, especially in LVe (FigII.10-13, 16-18). In MVe, most of the labeled neurons were concentrated in the magnocellular part (MVeMC); only a small number of neurons were located in the parvicellular part (MVePC).

**Trigeminal nucleus**
A few labeled neurons were found in the dorsomedial (Pr5DM) and ventrolateral (Pr5VL) parts of the principal sensory trigeminal nucleus on both sides. These neurons were either spindle shaped or triangular (FigII.9-10, 18). The labeled neurons in the oral spinal trigeminal nucleus (Sp5O) and the interpolar spinal trigeminal nucleus (Sp5I) were more numerous and more densely packed than in Pr5DM and Pr5VL. Most of these labeled neurons were large triangular or stellate cells with prominently labeled dendrites. They were concentrated in the ventral portion of these two nuclei (FigII.11-14, 18). Labeled neurons in the caudal spinal trigeminal nucleus (Sp5C) were smaller and less clustered than those in Sp5O and Sp5I, and were only found in the dorsal portion of this nucleus (FigII.15, 17-18).

At the level of rostral part of the motor trigeminal nucleus, a few small labeled neurons were observed in the space between the motor trigeminal nucleus (5N) and Pr5DM, possibly the area identified as parvicellular motor trigeminal nucleus, and in the area dorsal to 5N [probably the supratrigeminal nucleus (Su5)] on the ipsilateral side (FigII.9).

**The solitary nucleus**
Small to medium sized stellate neurons were labeled in the nucleus of solitary tract (Sol) with the majority present in the ventral (SolV) and lateral (SolL) parts of the nucleus on the ipsilateral side. The size of these neurons was smaller than those in the nearby spinal vestibular nucleus. In the caudal part of Sol, some labeled neurons were observed in the medial (SolM) and commissural (SolC) subdivisions (FigII.13-17).

**Cuneate and gracile nuclei**
Labeled neurons were observed in the ipsilateral cuneate (Cu) and gracile (Gr) nuclei, especially in their rostral parts. These labeled neurons were similar in appearance to those in the adjacent medullary reticular nucleus (FigII.14-17).

**Hindbrain reticular formation and related nuclei**
A prominent column of labeled neurons was found lateral to the rostral oral pontine reticular nucleus (PnO) and medial to the lateral lemniscus (ll); this region corresponds to the paralemniscal nucleus (PL) of Franklin and Paxinos (2008). These labeled neurons extended from the ventral portion of PL to the level dorsal to the superior cerebellar peduncle (scp).
Some of these labeled neurons appear to lie within the triangular nucleus of the lateral lemniscus (TrLL) and the medial paralemniscal nucleus (MPL) (FigII.7-8, 18). A small cell group dorsal to the rubrospinal tract (rs) contained a small cluster of labeled neurons; we identify this group as the epiarubrospinal nucleus (ERS) of Paxinos and Watson (2007). These labeled neurons were smaller than those in adjacent PnO (FigII.8).

**FigII.6** In this diagram of a coronal section through the caudal hindbrain at the level of the interpeduncular nucleus (IP), labeled neurons are shown in RMC, mainly in the ventral portion. Labeled neurons were present in mRt and RPC bilaterally. Labeled neurons were seen in LPAG and DMPAG on the ipsilateral side. A few labeled neurons were present in InC and Dk. A few labeled neurons were present in parts of the oculomotor complex (Su3C, MA3, EW). A small number of labeled neurons were present in DpG on the contralateral side. The photomicrograph in the upper right shows a small cluster of labeled cells in LPAG. The photomicrograph in the lower left shows large strongly labeled cells RMC.

Labeled neurons were observed in the lateral part of PnO, in an area medial to the pedunculotegmental nucleus (PTg). PTg also contained labeled neurons (FigII.7). In more caudal sections, labeled neurons in PnO were more medially placed. In the rostral PnO, labeled neurons were principally on the contralateral side; in the middle and caudal parts, labeled cells were principally on the ipsilateral side. Labeled cells in the caudal part of pontine reticular nucleus (PnC) appeared similar in size and shape to those in the caudal PnO. Labeled cells were present in the ventral part of pontine reticular nucleus (PnV) and they...
were similar to those large cells in the gigantocellular reticular nucleus (Gi) (Fig II.9 and 10). Most of the neurons in PnO, PnC, and PnV were large and strongly labeled.

Labeled neurons were found in the Kölliker-Fuse nucleus (KF), medial parabrachial nucleus (MPB), and lateral parabrachial nucleus (LPB). The neurons in KF were bilaterally labeled and those in MPB and LPB were mainly labeled ipsilaterally. Labeled neurons in these nuclei appeared similar in shape, but smaller than labeled neurons in Pr5 (Fig II.9 and 18).

**Fig II.7** In this diagram of a coronal section through the caudal hindbrain at the level of the pontine nuclei, labeled neurons are shown in the contralateral PL. In the reticular formation, labeled neurons were present in PnO and mRt with a contralateral predominance. Labeled neurons were present in the contralateral PTg. Labeled neurons were present in the ipsilateral PrCnF, VLPAG, LPAG, and Su3C. The photomicrograph in the upper right shows labeled cells in PrCnF. The photomicrograph in the lower left shows labeled cells in PL.

At the level of the rostral end of the fourth ventricle (4V), a dense cluster of labeled neurons was found in the ipsilateral Barrington’s nucleus (Bar). Lateral to Bar, a few labeled neurons were observed in the ipsilateral locus coeruleus (LC) (Fig II.10). Labeled neurons in the subcoeruleus nucleus extended from the alpha part of the subcoeruleus nucleus (SubCA) to the dorsal (SubCD) and the ventral (SubCV) parts of the subcoeruleus nucleus (Fig II.9). The labeling was predominantly ipsilateral. In sagittal sections, labeled neurons in the subcoeruleus nucleus are seen to form a crescent which partly surrounded 5N (Fig II.17).
small number of labeled neurons were observed in the contralateral A5 region (FigII.10). A few spindle shaped neurons were observed in the parvicellular reticular nucleus (PCRt) and the intermediate reticular nucleus (IRt) on both sides (FigII.10-18). In the caudal hindbrain, labeled neurons were concentrated in the caudal part of IRt between the dorsal (MdD) and ventral (MdV) parts of the medullary reticular nuclei, and were mainly in the ipsilateral IRt (FigII.13-15, 17). At the same level, a large number of labeled neurons were found in MdD and MdV with an ipsilateral predominance (FigII.14-17). Medial to MdV, a small number of neurons was labeled in the paramedian reticular nucleus (PMn) (FigII.14).

FigII.8 In this diagram of a coronal section through the caudal hindbrain at the level of the trochlear nucleus, labeled neurons are shown in the ipsilateral LPAG, VLPAG and adjacent PrCnF. In the reticular formation, labeled neurons were present in mRt and PnO bilaterally with a contralateral predominance. Labeled neurons were also present in the contralateral PL and ERS. The photomicrograph in the lower right shows large labeled cells in the ipsilateral PnO. The photomicrograph in the lower left shows labeled cells in PL and the adjacent ERS which has smaller neurons than PL.

At the level of the abducens nucleus (6N), a large number of labeled neurons were observed in Gi, the lateral paragigantocellular reticular nucleus (LPGi), the alpha part of the gigantocellular reticular nucleus (GiA), and the ventral part of the gigantocellular reticular nucleus (GiV). There were more labeled neurons on the ipsilateral side than the contralateral side (FigII.11-13, 16-17). In GiA and GiV, labeled neurons formed an arch on the ipsilateral
side covering the pyramidal tract (py) and the medial lemniscus (ml). Labeled neurons in the dorsal paragigantocellular reticular nucleus (DPGi) were predominantly contralateral (FigII.11-12). Ventral to LPGi, a small number of labeled neurons were observed in the parapyramidal nucleus (PPy) bilaterally (FigII.11). In some cases, a few labeled neurons also were found in the area ventral to the facial nucleus (7N), which is likely to correspond to the retrotrapezoid nucleus (RTz) (Smith et al. 1989) (FigII. 11).

FigII.9 In this diagram of a coronal section through the caudal hindbrain at the level of the rostral pole of LC, labeled neurons were mainly present in the ipsilateral MPB, LPB. Labeled neurons were present in bilateral Pr5VL, Su5, SubCD, and SubCV with an ipsilateral predominance. Labeled neurons were present in bilateral PnC but there were more neurons on the ipsilateral side. The photomicrograph in the lower right shows the band of labeled cells in SubCD and the photomicrograph in the lower left shows labeled cells in SubCV.

In the raphe, labeled neurons were found in the raphe magnus nucleus (RMg), raphe interpositus nucleus (RIP), raphe obscurus nucleus (ROb), and the raphe pallidus nucleus (RPa) (FigII.9-13). These labeled neurons were mostly oriented horizontally in coronal sections. They were smaller than the labeled neurons in PnC and Gi.

Lateral to the LPGi, a few labeled neurons were found in the rostroventrolateral reticular
nucleus (RVL) bilaterally (FigII.12). Dorsal to this nucleus, a small cluster of labeled neurons was found in the compact part of the nucleus ambiguus (AmbC). Ventral to AmbC, labeled neurons were observed in the Bötzinger complex (Bo), pre-Bötzinger complex (PrBo), and the rostral ventral respiratory group (RVRG) (FigII.12-14). More caudally, a few labeled neurons were observed in all parts of the contralateral retroambiguus nucleus (RAmb)

![Diagram of a coronal section through the caudal hindbrain at the level of the superior olive, labeled neurons are shown in a number of nuclei in the reticular formation (GiA, PnC, IRt, PCRt, LPGi), with an ipsilateral predominance. The densest group of labeled neurons is seen in the ipsilateral Gi and LPGi. In the raphe, labeled neurons were present in RIP, RMg, and RPa. Labeled neurons were present in the ventral part of Pr5 with an ipsilateral predominance. Labeled neurons were present bilaterally in MVe. Many labeled neurons were present in the ipsilateral Bar and a smaller number of neurons were present in the adjacent LC and SuVe. The photomicrograph in the upper right shows labeled cells in Bar and LC. The photomicrograph in the middle left shows labeled cells in IRt. The photomicrograph in the lower right shows labeled cells in Pr5VL.](FigII.10)

**FigII.10** In this diagram of a coronal section through the caudal hindbrain at the level of the superior olive, labeled neurons are shown in a number of nuclei in the reticular formation (GiA, PnC, IRt, PCRt, LPGi), with an ipsilateral predominance. The densest group of labeled neurons is seen in the ipsilateral Gi and LPGi. In the raphe, labeled neurons were present in RIP, RMg, and RPa. Labeled neurons were present in the ventral part of Pr5 with an ipsilateral predominance. Labeled neurons were present bilaterally in MVe. Many labeled neurons were present in the ipsilateral Bar and a smaller number of neurons were present in the adjacent LC and SuVe. The photomicrograph in the upper right shows labeled cells in Bar and LC. The photomicrograph in the middle left shows labeled cells in IRt. The photomicrograph in the lower right shows labeled cells in Pr5VL.

**Cerebellar nuclei**

Intensely labeled neurons were observed in the contralateral anterior interposed cerebellar nucleus (IntA), posterior interposed cerebellar nucleus (IntP), and medial cerebellar nucleus (Med), including the dorsal lateral protuberance (MedDL) (FigII.11-12, 16-18). The intensity
Estimates of the number of labeled cells
The estimates of the number of labeled cells in each nucleus or area are shown in Table II.1 and the following bar graph. The data for two animals are presented. While a sample of two has limitations, it is notable that the counts for large areas do not vary by more than 10% between the two cases. The total counts (all areas, contralateral and ipsilateral) for the two cases are almost identical. The counts show that the contribution of the motor and somatosensory cortex is only about 25% of the total, whereas the brainstem tegmental and

FigII.11 In this diagram of a coronal section through the caudal hindbrain at the level of 7N, labeled neurons are shown in a number of nuclei in the reticular formation (GiA, Gi, IRT, PCRtA, LPGi, DPGi), with an ipsilateral predominance except in DPGi. The densest group of labeled neurons is seen in the ipsilateral GiA and LPGi. Labeled neurons were present in the ventral portion of Sp5O bilaterally. In the raphe, labeled neurons were present in RMg and RPa. Labeled neurons were present in MVe (bilateral), and in LVe and SuVe (ipsilaterally). Labeled neurons were present in Med and IntA on the contralateral side. The photomicrograph in the upper right shows large strongly labeled cells in LVe. The photomicrograph in the lower right shows two labeled cells in RTz. The photomicrograph in the lower left shows a band of large labeled cells in GiA. The photomicrograph in the upper left shows strongly labeled cells in IntA.
reticular nuclei account for over 50% of the total. The contribution from the red nucleus was about 7% of the total.

**Discussion**

This study confirms that the overall pattern of origin of descending spinal tracts in the mouse is similar to that found in other species (Hayes and Rustioni 1981; Leong et al. 1984; Carlton et al. 1985; Gross and Oppenheim 1985; Nudo and Masterton 1988; Carretta et al. 2001). The present study has revealed a number of sites of origin of descending spinal tracts that were

**FigII.12** In this diagram of a coronal section through the caudal hindbrain at the level of the rostral part of Amb, labeled neurons are shown in a number of nuclei in the reticular formation (GiV, Gi, IRt, PCRt, LPGi, LPGiE, DPGi), with an ipsilateral predominance except in DPGi. The densest group of labeled neurons is seen in the ipsilateral GiV. Labeled neurons were present in the ventral part of Sp5I bilaterally. In the raphe, labeled neurons were present in RPa. Labeled neurons were present in MVe and SpVe (bilaterally). Labeled neurons were present in Med, MedDL, and IntP on the contralateral side. Labeled neurons were present bilaterally in Amb and adjacent respiratory nuclei (Bo, RVL) with a contralateral predominance. The photomicrograph in the upper right shows large strongly labeled cells in MVeMC. The photomicrograph in the lower right shows large strongly labeled cells in Gi. The photomicrograph in the lower left shows a cluster of strongly labeled cells in Amb. The photomicrograph in the upper left shows strongly labeled cells in IntP.
previously unrecognized in the mouse. The major significance of this report is that it provides a much-needed baseline for study of recovery of descending tracts after experimental spinal cord injury in the mouse.

**Ipsilateral versus contralateral projections**

In our experiments, we attempted to make very large injections in order to identify all cell groups that project to the spinal cord. Because of this, there was a small amount of spread across the midline in a number of cases. This means that the distinction between ipsilateral

![Diagram of a coronal section through the caudal hindbrain at the level of rostral 12N. Labeled neurons were present in bilateral MVe, SpVe, and Sp5I. Labeled neurons were present in a number of subnuclei of the ipsilateral Sol (SolV, SolL, SolM). In the reticular formation, labeled neurons were present in bilateral PCRt, IRt, Gi, GiV, LPGi with an ipsilateral predominance in GiV and LPGi, contralateral dominance in Gi. The densest group was GiV. Labeled neurons were present in bilateral Amb and PrBo with a contralateral predominance. Labeled neurons were present in ROb and RPa. The photomicrograph in the lower right shows strongly labeled cells in ROb and adjacent GiV. The photomicrograph in the lower left shows large strongly labeled cells in Sp5I.**

and contralateral labeling in our data was somewhat compromised. While we would have preferred to have strictly unilateral injections, our main aim was to label as many cell groups as possible, and we therefore were prepared to accept a small blurring of the distinction.
between ipsilateral and contralateral origins.

**Cerebral cortex**

We found many labeled cells in the contralateral motor and the somatosensory areas. This is consistent with previous studies on the origin of the corticospinal tract in mammals (Hayes and Rustioni 1981; Miller 1987; Casale et al. 1988; Nudo and Masterton 1990; Masson et al. 1991; Rathelot and Strick 2006). The exception is the hedgehog, in which the majority of labeled neurons are observed in the ipsilateral cortex (Michaloudi et al. 1988). Most of the

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**FigII.14** In this diagram of a coronal section through the caudal hindbrain at the level of the inferior olivary nucleus (IO), labeled neurons are shown in a number of nuclei in the reticular formation (MdV, MdD, IRt, PMn), with an ipsilateral predominance. Labeled neurons were present in the ventral part of Sp5I bilaterally. Labeled neurons were present bilaterally in Amb and adjacent respiratory nuclei (RVRG) with a contralateral predominance. Labeled neurons were present in a number of subnuclei of Sol (SoIL and SoIV). A small number of neurons were present in Cu. The photomicrograph in the upper right shows labeled cells in Cu. The photomicrograph in the lower right shows large strongly labeled cells in MdD. The photomicrograph in the lower left shows smaller labeled cells in PMn and larger labeled cells in MdV.

labeled cells we identified are large pyramidal neurons in layers 5 and 6. A small number of labeled neurons were found in the ipsilateral motor and somatosensory areas. This might
represent the origin of ipsilateral corticospinal tract, but it is also possible that some of these cells were labeled by the small amount of retrograde tracer that spread across the midline of the spinal cord in some cases. From the publication of Nudo and Masterton in 1990, it is expected that the ipsilateral corticospinal neurons account for less than 10% of the total corticospinal neurons in the mouse. However, one anterograde study on the monkey by Dum and Strick in 1996 showed that the ipsilateral corticospinal fibers vary from for 10-23% of the total corticospinal fibers according to their location in the cortex. Bareyre et al reported the number of corticospinal fibers in the mouse: 10,997±833 in the dorsal corticospinal tract, 390±44 in the dorsolateral tract, and 191±18 in the ventral tract. But this transgenic study

FigII.15 In this diagram of a coronal section through the caudal hindbrain at the level of the pyramidal decussation (pyx), labeled neurons are shown in a number of nuclei in the reticular formation (MdV, MdD, IRt), with an ipsilateral predominance. Labeled neurons were present in the dorsal portion of Sp5C bilaterally. Labeled neurons were present contralaterally in RAmb. Labeled neurons were present in a number of subnuclei of Sol (SolC, SolM). A small number of neurons were present in the ventral part of Cu and Gr. The photomicrograph in the lower right shows a large number of labeled cells in IRt. The photomicrograph in the lower left shows a cluster of strongly labeled cells in RAmb.
could not differentiate the ipsilateral fibers from the contralateral ones. We found that many labeled neurons were present in M1, S1FL, S1HL, and S1Tr of the somatosensory cortex. This is consistent with the findings of Li et al (1990) and Tracey (2004) in rats. Neurons in the parietal association cortex (PtA), secondary visual cortex (V2), insular cortex, and prefrontal cortex have been shown to project to the spinal cord in the rat (Miller 1987). However, we did not find labeled cells in these areas. We estimate that there are about 13,350 labeled cells in the motor cortex (M1 plus M2) and about 9,270 labeled cells in the somatosensory cortex (all S1 areas plus S2) (see TableII.2). When compared with the number of labeled neurons in the red nucleus and hindbrain reticular formation (around 58,190), the motor and somatosensory cortex contribution (22,620) is not a large component of the total descending projection to the spinal cord (see TableII.3). This is consistent with a view that the corticospinal fibers arising in the motor cortex are not a major contributor to

FigII.16 In this diagram of a sagittal section close to the midline, labeled neurons are seen in the hindbrain, cerebellum, midbrain, diencephalon, hypothalamus and cerebral cortex. In the hindbrain reticular formation, labeled cells are present in PnC, Gi, LPGi, GiV, IRT, and MdV. In the dorsal part of the hindbrain, labeled cells are present in Bar, MVe, Sol, and rostral Cu. In the cerebellum, labeled cells are present in Med. In the midbrain, labeled cells are concentrated in the red nucleus (RMC, RPC), mRt, and the PAG (DLPAG). In the diencephalon, labeled neurons are present in PCom, MCPC, PF, RPF, H. In the hypothalamus, labeled neurons are present in the perifornical part of lateral hypothalamus (PeFLH), VMH, RChL, PaPo. In the cerebral cortex, labeled neurons are present in M2. The photomicrograph in the upper right shows labeled cells in Med. The photomicrograph in the lower right shows large strongly labeled cells in PnC. The photomicrograph in the lower left shows a cluster of small labeled cells in PaPo.
motor control in mice (Watson and Harvey 2009). Moreover, it has been shown that about 90% of corticospinal fibers are distributed in the dorsal horn and intermediate laminae of the spinal cord, with a small amount of fibers from the dorsolateral and ventral corticospinal tracts terminating on lamina 9 (Bareyre et al. 2005). In terms of the direct projections from the cortex to the spinal cord motoneurons, they have been shown in monkeys (Porter and Lemon 1993; Lemon 2008). But they are not demonstrated by the electrophysiological study in rats (Alstermark et al., 2004). In Bareyre’s paper (2005), the monosynaptic connections are illustrated by overlapping the corticospinal collaterals with a synaptic marker, but it might need to be confirmed with electron microscopy. This reinforces the argument that the mouse corticospinal tract is an insignificant player in motor control, compared to the role of this tract in primates such as the rhesus monkey, in which most of the corticospinal fibers terminate in lamina 7 and 9 of the ventral horn (Dum and Strick 1996). Given its extensive termination in the dorsal horn, the mouse corticospinal tract may be more involved in modulating sensory information from the spinal cord rather than the control of limb movement. This means that the role of corticospinal fibers in the recovery of movement after spinal cord injury must be interpreted with caution.

**Hypothalamic and diencephalic areas**

Descending projections from the hypothalamus and diencephalon have been identified in a wide range of mammalian and non-mammalian vertebrates. The origin of these neurons varies between non-mammalian classes of vertebrates, but the descending projections most commonly arise from a homologue of the paraventricular nucleus and at least one cell group in the ventral thalamus (Smeets and Timerick 1981; Berk and Finkelstein 1983; Prasada Rao et al. 1987; Masino and Knudsen 1992; Rao et al. 1993; New et al. 1998; Cruce et al. 1999; Sanchez-Camacho et al.2002; Barreiro-Iglesias et al. 2008). In common with a number of studies in mammals (Basbaum and Fields 1979; Sawchenko and Swanson 1982; Leong et al. 1984a; Holstege 1987a; Masson et al. 1991; Hallbeck and Blomqvist 1999; Hallbeck 2000; Kc et al. 2002), we found labeled neurons in the ipsilateral paraventricular hypothalamus, the medial part of ZID, and in LH and PH. A small number of weakly labeled neurons were found in DM. We also found labeled neurons in a number of caudal diencephalic nuclei, notably PF and SPF. These latter findings are consistent with those of Schwanzel-Fukuda et al (1984), Nudo and Masterton (1988), Takada (1993), and Marini et al (1999). Laterally, a few labeled neurons are seen in the ipsilateral subthalamic nucleus in the present thesis. However, spinal projections from the subthalamic nucleus have only been reported in the
monkey after upper cervical injections of the retrograde tracer (Takada et al., 1987; Mizuno et al., 1988) and the majority of labeled neurons in the monkey are on the contralateral side. This inconsistency needs to be solved with anterograde tracer injections to the subthalamic nucleus.

Nuclei of the pretectal area (including MCPC, PCom, InC, and Dk) have been shown to project to the spinal cord in a range of different vertebrates (Castiglioni et al. 1978; Crutcher et al. 1978; Leong et al. 1984a; Carlton et al. 1985; Gross and Oppenheim 1985; Nudo and Masterton 1988; Masino and Knudson 1992; Crique et al. 1999; de Boer-van Huizen and ten Donkelaar 1999; Sanchez-Camacho et al. 2001a; Satoda et al. 2002). We found many labeled neurons in ipsilateral InC, Dk, and EW, and a small number of neurons in PCom (bilateral) and the contralateral MCPC. This is consistent with the findings cited above. Developmentally, these pretectal nuclei belong to prosomere 1 of diencephalon (Puelles et al. 2007), therefore they are discussed here.

**Red nucleus**

The red nucleus sends a major tract to the contralateral spinal cord in mammalian and non-mammalian vertebrates (Kuypers et al. 1962; Poirier and Bouvier 1966; Warner and Watson 1972; Miller and Strominger 1973; Castiglioni et al. 1978; Crutcher et al. 1978; Wild et al. 1979; Smeets and Timerick 1981; Huismann et al. 1982; Carlton et al. 1985; Okado and Oppenheim 1985; Prasada Rao et al. 1987; Nudo and Masterton 1988; Masino and Knudson 1992; New et al. 1998; Crique et al. 1999; Carretta et al. 2001; Satoda et al. 2002; Tsukamoto et al. 2003; Chiocchetti et al. 2006; VanderHorst and Ulfhake 2006; Stockx et al. 2007; Warren et al. 2008). As with the corticospinal projection (see above), the exception is the hedgehog, in which the ipsilateral projection is larger than the contralateral (Kunzle 1992). The neurons of RMC in mammals are topographic organized, with neurons projecting to the cervical cord dorsomedially placed, and those projecting to the lumbar cord ventrolaterally placed (Holstege and Tan 1988). An ipsilateral rubrospinal component has also been identified (Martin and Dom 1970; Warner and Watson 1972; Shieh et al. 1983; Holstege 1987b; Holstege and Tan 1988; Michaloudi et al. 1988; Kunzle 1992). Consistent with the majority of mammalian studies cited above, we found many labeled neurons in the contralateral RMC, and a few labeled neurons in RPC. According to our estimates, the red nucleus projection to the spinal cord represents about 7% of the total (as measured by comparing the number of labeled cells in the red nucleus with the total number of labeled
Fig II.17 In this diagram of a sagittal section lateral to the mammillothalamic tract (mt), labeled neurons are seen in the hindbrain, cerebellum, midbrain, diencephalon, hypothalamus and cerebral cortex. In the hindbrain reticular formation, densely labeled neurons are present in SubCD, SubCV, MdD, MdV, and LPGi; less densely labeled neurons are present in PCrT, IRt, PnO. In the dorsal part of the hindbrain, densely labeled neurons are present in MVeMC and caudal SpVe; less densely labeled neurons are present in PTg, MPB, LC, MVePC, caudal Sol, and rostral Cu. In the cerebellum, labeled neurons are present in MedDL, IntA, and IntP. In the midbrain, labeled cells are concentrated in RMC. Labeled neurons are also present in mRt, DpWh, PrCnF. In the diencephalon, labeled neurons are seen in H. In the hypothalamus, labeled neurons are present in LH (PeFLH, PLH). In the cerebral cortex, labeled neurons are present in both M1 and M2. The photomicrograph in the upper right shows a cluster of strongly labeled cells in caudal SpVe. The photomicrograph in the upper left shows a stripe of labeled cells in mRt and PrCnF. The photomicrograph in the lower left shows large strongly labeled cells in RMC.

Tectum
A projection from the midbrain tectum (SC in mammals) to the contralateral spinal cord is common to a variety of vertebrates that have been studied (Altman and Carpenter 1961; Nyberg-Hansen 1964a; Martin 1969; Kuypers and Maisky 1975; Graham 1977; Harting 1977; Basbaum and Fields 1979; Hayes and Rustioni 1981; Huerta and Harting 1982; Leong et al. 1984; Carlton et al. 1985; Nudo and Masterton 1988; Masson et al. 1991; Olivier et al. 1991; Cruce et al. 1999; Satoda et al. 2002). However, it is notable that ten Donkelaar (1976) did not find tectospinal projections in three reptile species he studied. Previous studies in mammals found that the tectospinal neurons were mainly located in the intermediate gray layer of the superior colliculus (InG), but were also present in DpG(e.g. Nudo and Masterton,
This pattern was confirmed by our tracing studies. In our material, the long axis of the spinally-projecting neurons was parallel to the layers of SC, whereas in the monkey.

**Fig II.18** In this diagram of a sagittal section at the lateral edge of SC, labeled neurons are seen in the hindbrain, cerebellum, midbrain, diencephalon, extended amygdala, and cerebral cortex. In the hindbrain, labeled neurons are present in the ventral portion of Pr5, Sp5O, and Sp5I and in the dorsal portion of Sp5C. A few labeled neurons are present in RVL and PCRTA. In the dorsal hindbrain, labeled neurons are present in SuVe, LVe, MVe, and SpVe. In the rostral hindbrain, a large number of labeled neurons are present in PL. A few labeled neurons are also present in KF. In the cerebellum, labeled neurons are present in both IntA and IntP. In the midbrain, a few labeled cells are present mRT. In the diencephalon, a number of neurons are present in ZID. In the amygdala, a small number of labeled neurons are present in the extended amygdala (EA). In the cerebral cortex, a large number of labeled neurons are present in M1, S1HL, S1Sh, S1Tr. The photomicrograph in the upper right shows large strongly labeled cells in SpVe. The photomicrograph in the lower right shows a stripe of labeled cells in PL and adjacent KF. The photomicrograph in the lower left shows a band of strongly labeled cells in ZID.

tectospinal neurons are aligned perpendicular to the collicular layers (Castiglioni et al. 1978). The number of cells giving rise to tectospinal tract fibers in mammals is surprisingly small (Nudo and Masterton 1989); carnivores had the largest number of spinally projecting cells in the contralateral SC (628 in the raccoon and 909 in the cat), but in 7 species of primates studied the number of spinally projecting cells averaged only 220. The average for 23 non-carnivore mammals studied was 243. On the basis of our counts in the mouse, we estimate that there are about 160 spinally projecting cells in the superior colliculus. Nudo and Masterton (1989) suggested that the influence of the tectum on neck movement may be chiefly mediated by tectal projections to hindbrain nuclei, which in turn project to the
cervical spinal cord.

**Table II.1 Estimates of the numbers of labeled neurons in each nucleus in the brain**
(cells were counted in every seventh section so the raw count has been multiplied by 7)

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</table>
Other midbrain nuclei

The midbrain PAG has been shown to project to the spinal cord in a number of mammals (Castiglioni et al. 1978; Hayes and Rustioni 1981; Mantyh 1983; Carlton et al. 1985; Nudo and Masterton 1988; Masson et al. 1991; Cowie and Holstege 1992; Kunzle 1992; Satoda et al. 2002; VanderHorst and Ulfhake 2006). In most cases the projection was found to be ipsilateral (Castiglioni et al. 1978; Mantyh 1983; Carlton et al. 1985; Nudo and Masterton 1988; Cowie and Holstege 1992; VanderHorst and Ulfhake 2006), but Hayes and Rustioni (1981) identified a contralateral projection arising from LPAG, and others found a bilateral projection (Masson et al. 1991; Kunzle 1992; Satoda et al. 2002). We found labeled neurons in the ipsilateral LPAG, VLPAG, and DMPAG. Some labeled neurons were also observed in the pretectal PAG at the rostral level of the red nucleus. In this area, labeled neurons form a small cluster which is adjacent to Lth. Our result is consistent with most of studies on
mammals as mentioned above.

Spinal projecting neurons have been found in the mesencephalic trigeminal nucleus (Me5) (Matsushita et al. 1981; Leong et al. 1984; Michaloudi et al. 1988; Nudo and Masterton 1988; Sanchez-Camacho et al. 2001a), but we did not observe labeled neurons in this nucleus in our experiments. We did observe a number of labeled neurons in bilateral mRt, a finding which has been previously reported by other studies (Leong et al. 1984; Michaloudi et al. 1988; Webster and Steeves 1988; Hassouna et al. 2001).

Table II.2 The estimated total numbers of some nuclei (cells were counted in every seventh section so the raw count has been multiplied by 7).

<table>
<thead>
<tr>
<th>brain areas</th>
<th>Ipsilateral (No.1)</th>
<th>Contralateral (No.1)</th>
<th>total</th>
<th>Ipsilateral (No.2)</th>
<th>Contralateral (No.2)</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor cortex</td>
<td>4564</td>
<td>8099</td>
<td>12663</td>
<td>5145</td>
<td>8897</td>
<td>14042</td>
</tr>
<tr>
<td>Somatosensory cortex</td>
<td>3346</td>
<td>5488</td>
<td>8834</td>
<td>4018</td>
<td>5691</td>
<td>9709</td>
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<tr>
<td>Red nucleus</td>
<td>2086</td>
<td>3955</td>
<td>6041</td>
<td>1960</td>
<td>4480</td>
<td>6440</td>
</tr>
<tr>
<td>Hindbrain nuclei</td>
<td>31087</td>
<td>20825</td>
<td>51912</td>
<td>31487</td>
<td>20513</td>
<td>52000</td>
</tr>
<tr>
<td>Other nuclei</td>
<td>7287</td>
<td>3584</td>
<td>10871</td>
<td>8253</td>
<td>3500</td>
<td>11753</td>
</tr>
<tr>
<td>Total</td>
<td>48370</td>
<td>41951</td>
<td>90321</td>
<td>50863</td>
<td>43081</td>
<td>93944</td>
</tr>
</tbody>
</table>

Table II.3 The estimated total numbers of some nuclei (cells were counted in every seventh section so the raw count has been multiplied by 7).

<table>
<thead>
<tr>
<th>brain area</th>
<th>total number (No. 1)</th>
<th>Percentage of total number (No. 1)</th>
<th>Total number (No. 2)</th>
<th>Percentage of total number (No. 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>21497</td>
<td>23.8%</td>
<td>23751</td>
<td>25.3%</td>
</tr>
<tr>
<td>Red nucleus</td>
<td>6041</td>
<td>6.7%</td>
<td>6440</td>
<td>6.9%</td>
</tr>
<tr>
<td>Hindbrain nuclei</td>
<td>51912</td>
<td>57.5%</td>
<td>52000</td>
<td>55.3%</td>
</tr>
<tr>
<td>Other nuclei</td>
<td>10871</td>
<td>12.0%</td>
<td>11753</td>
<td>12.5%</td>
</tr>
<tr>
<td>Total</td>
<td>90321</td>
<td>100%</td>
<td>93944</td>
<td>100%</td>
</tr>
</tbody>
</table>
Vestibular nuclei
Two main vestibulospinal tracts, the lateral and the medial, have been described. The lateral tract arises from the ipsilateral LVe and is driven by the saccule and utricle (Nyberg-Hansen 1964b; Peterson and Coulter 1977; Basbaum and Fields 1979; Hayes and Rustioni 1981; Leong et al. 1984; Carlton et al. 1985; Michaloudi et al. 1988; Nudo and Masterton 1988; Masson et al. 1991; Masino and Knudson 1992; Wada et al. 1993; Sato et al. 1996; Sato et al. 1997); it extends the full length of the spinal cord (Hayes and Rustioni 1981; Wada et al. 1993). The medial vestibulospinal tract arises from MVe and SpVe bilaterally, and is driven by the semicircular canals (Nyberg-Hansen 1964b; Akaike et al. 1973; Carleton and Carpenter 1984; Cox and Peusner 1990). SuVe has also been found to contain spinal projecting neurons, and these cells are concentrated in the caudal or ventral portion of this nucleus (Leong et al. 1984; Kitao et al. 1993). Consistent with these previous studies, we found medium to large labeled neurons in MVe, SpVe, and LVe, and a few labeled cells in SuVe.

A third vestibulospinal tract, the caudal vestibulospinal tract, has been described by Peterson et al (Peterson et al. 1978). They showed that it arises from the caudal MVe, SpVe, and vestibular group F. However, a more recent study argues that it originates only from the caudal MVe (Bankoul and Neuhuber 1992). In our study, labeled cells were found in the caudal MVe and the caudal SpVe, but labeled cells were not found in the vestibular group F.

The solitary nucleus
This nucleus is an important visceral sensory center related to such functions as swallowing, respiration function, and sensation from the internal organs (Jean 1972; Ogawa et al. 1984;
Pantaleo and Corda 1986). Consistent with its role in respiratory regulation, it has been shown to project to the phrenic motor nucleus in the cervical spinal cord and to ventral horn at thoracic levels (Crutcher et al. 1978; Kneisley et al. 1978; Loewy and Burton 1978; Smeets and Timerick 1981; Leong et al. 1984; Michaloudi et al. 1988; Mtui et al. 1993; Cruce et al. 1999; Stockx et al. 2007). The solitariospinal tract has been shown to reach lumbar and sacral segments (Carlton et al. 1985; Michaloudi et al. 1988; Masson et al. 1991). In previous studies, most of the spinal projecting neurons were found to be medium sized cells intermingled with a few large neurons in the ventral and ventrolateral subdivisions (Loewy and Burton 1978; Rikard-Bell et al. 1984; Nudo and Masterton 1988; Masson et al. 1991; Mtui et al. 1993; Sanchez-Camacho et al. 2001a). The medium sized cells of the ventrolateral subdivision (SolVL) were shown to project mainly to the cervical spinal cord, while the large SolVL neurons project mainly to the thoracic spinal cord (Loewy and Burton 1978). Smaller spinal projections were found to arise from the intermediate subdivision (SolI) and the commissural subdivision (SolC) (Loewy and Burton 1978; Leong et al. 1984; Mtui et al. 1993). We found labeled cells in the nucleus of the solitary tract on both sides, with an ipsilateral predominance. The majority of labeled neurons were observed in SolV, SolL on the ipsilateral side. Some labeled neurons were observed in the caudal SolM and SolC of this nucleus. These neurons were small to medium sized and lightly labeled compared with labeled neurons in the nearby reticular formation.

The sensory trigeminal nuclei of the hindbrain
We found labeled cells in Pr5 and all parts of spinal trigeminal nucleus. The distribution was bilateral, but there was an ipsilateral predominance in Pr5 and Sp5C. In sagittal sections, labeled neurons in Pr5, Sp5O, and Sp5I are seen to form a continuous rostrocaudal band in the ventral part of the trigeminal complex. A stripe of labeled cells was observed in the area between Pr5 and 5N. This area has been identified as the intertrigeminal area (Int5) (Chamberlin and Saper 1998; Radulovacki et al. 2003; Song et al. 2006), but it may overlap with the parvicellular trigeminal nucleus of Franklin and Paxinos (2008). Cells dorsal to Int5 appear to lie in Su5. Many previous studies have shown that Sp5O, Sp5I, and Sp5C give rise to axons which reach the spinal cord (Kuypers and Maisky 1975; Craig 1978; Burton et al. 1979; Matsushita et al. 1981; Ruggiero et al. 1981; Leong et al. 1984; Gross and Oppenheim 1985; Phelan and Falls 1991; Masino and Knudson 1992; Diagne et al. 2006). Our results are consistent with these studies. As noted above, Me5 has also been identified as the source of descending fibers to the spinal cord (Matsushita et al. 1981), but we did not find labeled cells
in this nucleus.

**Locus coeruleus and the subcoeruleus area**

Ipsilateral spinal projections from LC have been documented in a variety of vertebrate species (Hancock and Fougerousse 1976; Basbaum and Fields 1979; Guyenet 1980; Hayes and Rustioni 1981; Leong et al. 1984; Carlton et al. 1985; Fritschy et al. 1987; Michaloudi et al. 1988; Clark and Proudfoot 1991; Sanchez-Camacho et al. 2001a; Sanchez-Camacho et al. 2001b; VanderHorst and Ulfhake 2006). We found labeled cells in the ventral portion of the ipsilateral LC, but these cells were not as densely packed as those labeled cells in the adjacent Bar (Russo et al. 2004). A few lightly labeled cells were seen in the contralateral LC. The adjacent subcoeruleus area has also been shown to contain spinal projecting neurons (Kneisley et al. 1978; Hayes and Rustioni 1981; Leong et al. 1984; Carlton et al. 1985; Okado and Oppenheim 1985; Tsukamoto et al. 2003; VanderHorst and Ulfhake 2006). Our results are consistent with these studies. We found labeled neurons in the subcoeruleus nucleus bilaterally with an ipsilateral predominance. These labeled neurons are smaller than those in PnO. In sagittal sections, the band of labeled cells forms a characteristic crescent along the rostral border of 5N.

**Cerebellar nuclei**

The cerebellospinal pathway has been shown to originate from the contralateral Med and interposed cerebellar nuclei (IntA, IntP) (Batton et al. 1977; Bangma et al. 1984; Leong et al. 1984; Gross and Oppenheim 1985; Nudo and Masterton 1988; Arends and Zeigler 1991; Sanchez-Camacho et al. 2001a). We found many labeled neurons in IntA, IntP, and Med on the contralateral side.

**Gracile and cuneate nuclei**

We found a small number of labeled cells in Cu and Gr, which is consistent with previous reports on spinal projections from these nuclei in the rat, hedgehog, cat, and monkey (Burton and Loewy 1977; Carlton et al. 1985; Michaloudi et al. 1988; Kudo et al. 1993). In our study, most of the labeled neurons were small to medium sized and located in the rostroventral part of these two nuclei.

**Reticular nuclei of the hindbrain**

We consider the reticular formation of the hindbrain to be made up of a medial magnocellular column (chiefly PnO, PnC, Gi, MdV) and a lateral parvicellular column (PCRt, PCRtA,
MdD), separated by an intermediate nucleus (IRt). We do not consider the precerebellar nuclei, LC, or the raphe nuclei to be integral parts of these reticular columns because these former groups are developmentally and functionally distinct. The respiratory neuron groups maybe specialized part of the reticular formation, we will consider them separately.

The magnocellular nuclei of the reticular formation have been shown to give rise to major projections to the spinal cord in a wide variety of vertebrate species (Crutcher et al. 1978; Kneisley et al. 1978; Basbaum and Fields 1979; Zemlan and Pfaff 1979; Goode et al. 1980; Hayes and Rustioni 1981; Martin et al. 1982; Leong et al. 1984; Carlton et al. 1985; Oka et al. 1986; Sirkin and Feng 1987; Michaloudi et al. 1988; Nudo and Masterton 1988; Shen et al. 1990; Holstege 1991; Kausz 1991; Masson et al. 1991; Hobbelen et al. 1992; Kudo et al. 1993; Wada et al. 1993; Aicher et al. 1995; Cruce et al. 1999; de Boer-van Huizen and ten Donkelaar 1999; Carretta et al. 2001; Sanchez-Camacho et al. 2001; Stockx et al. 2007; Reed et al. 2008). While nomenclatural inconsistencies abound, we consider that the magnocellular reticular nuclei should be taken to include PnO, PnC, PnV, Gi (including GiA, GiV), DPGi, and LPGi.

We found a large number of labeled neurons in bilateral PnO and PnC. Most of labeled neurons were larger than those labeled neurons in the subcoeruleus nucleus. Labeled neurons were found to be closer to the midline in more caudal sections than they were in rostral sections. We also observed a very large number of labeled neurons in Gi (GiA, GiV, Gi). Labeled neurons in GiA and GiV are predominantly ipsilaterally located and form an arch with neurons in raphe nuclei and LPGi. Labeled neurons in the dorsal part of Gi are more sparsely distributed with a contralateral dominance. Some labeled neurons in Gi are larger than those in GiA and GiV. A few labeled neurons were observed in DPGi. These findings are consistent with other studies of the gigantocellular nuclei (Kneisley et al. 1978; Zemlan and Pfaff 1979; Hayes and Rustioni 1981; Newman et al. 1983; Leong et al. 1984; Carlton et al. 1985; Metcalfe et al. 1986; Oka et al. 1986; Prasada Rao et al. 1987; Glover and Petursdottir 1988; Webster and Steeves 1988; Shen et al. 1990; Kausz 1991; Masson et al. 1991; Webster and Steeves 1991; Rao et al. 1993; Wada et al. 1993; Aicher et al. 1995; New et al. 1998; Cruce et al. 1999; Carretta et al. 2001; Gahtan and O’Malley 2003; Tsukamoto et al. 2003), and almost identical to the findings of VanderHorst and Ulfhake (2006) in the mouse.

It has been reported that PCRT and IRt have spinal projecting neurons (Gross and Oppenheim
1985; Nudo and Masterton 1988; Stockx et al. 2007). We found a few neurons in these two nuclei bilaterally. In the caudal portion of IRt, the density of labeled neurons increases, and in the most caudal hindbrain sections, they form a band that separates MdV and MdD. At the same level, a large number of labeled neurons were seen in both MdD and MdV with an ipsilateral predominance. Labeled neurons in each of these two nuclei form a band which is parallel to the band of labeled neurons in IRt. In the dorsal portion of MdD, IRt, and MdV, labeled cells are more numerous in the more caudal regions. These findings are consistent with similar studies in other mammals (Peterson et al. 1975; Hayes and Rustioni 1981; Leong et al. 1984; Carlton et al. 1985; Leite-Almeida et al. 2006).

The ambiguous nucleus and the hindbrain respiratory nuclei

We found labeled cells in nucleus ambiguus and related respiratory nuclei. In the AmbC, the labeled cells form a small cluster bilaterally with a contralateral predominance. This is consistent with other reports (Leong et al. 1984; Gross and Oppenheimer 1985; Nudo and Masterton 1988; Lan et al. 1997; Ellenberger 1999). Ventral to Amb, labeled neurons were found in RVL, Bo, PrBo, and RVRG. Labeled cells in these latter nuclei are not as densely packed as AmbC: a finding which is consistent with results of previous studies (Kausz 1991; Mtui et al. 1995; Lan et al. 1997; Ellenberger 1999; Buhler et al. 2004; Russo et al. 2005). Cells of the contralateral RAmb are labeled but the density of labeled cells is much less than that of Amb. This is consistent with reports of studies in cat, rat, and mouse (Hardy et al. 1998; Gerrits et al. 2000; VanderHorst 2005; Boers et al. 2006).

Raphe nuclei

The raphe nuclei have been reported to project to the spinal cord in a variety of species (Kneisley et al. 1978; Leichnetz et al. 1978; Basbaum and Fields, 1979; Zemlan and Pfaff 1979; Hayes and Rustioni 1981; Smeets and Timerick 1981; ten Donkelaar et al. 1981; ten Donkelaar and de Boer-van Huizen 1982; Leong et al. 1984; van Mier and ten Donkelaar 1984; Gross and Oppenheimer 1985; Okado and Oppenheimer 1985; Edwards et al. 1987; Holstege and Tan 1987; Prasada Rao et al. 1987; Michaloudi et al.1988; Nudo and Masterton 1988; Webster and Steeves 1988; Shen et al. 1990; Kausz 1991; Masson et al. 1991; Hobbelen et al. 1992; Kudo et al. 1993; Rao et al. 1993; Gilbey et al. 1995; New et al. 1998; Adli et al, 1999; Carretta et al. 2001; Sanchez-Camacho et al. 2001a; VanderHorst and Ulfhake 2006). We found labeled neurons in RMg, RPa, ROB, and RIP. The long axis of most labeled neurons is oriented horizontally and they are as strongly labeled as those neurons in adjacent Gi and PnC. This is consistent with results from previous studies as
Novel sites of origin of descending spinal fibers
We found labeled cells in two hindbrain nuclei that have not previously been shown to project to the spinal cord. They are PrCnF and ERS. The rostral part of PrCnF lies between SC dorsally and mRt ventrally, the caudal part of PrCnF lies between IC dorsally and the microcellular tegmental nucleus (MiTg) ventrally (Franklin and Paxinos, 2008). The nucleus has a distinct outline in acetylcholinesterase sections showing in plate 70 of Franklin and Paxinos (2008). This nucleus has not previously been reported to project to the spinal cord.

One study found that a large number of neurons projected to the spinal cord from the medial part of the cuneiform nucleus (CnF) in the monkey, but these authors did not mention PrCnF (Castiglioni et al. 1978). In the cat, labeled neurons reported to be in the medial part of CnF after cervical spinal cord injections (Satoda et al. 2002). It is possible that some of these labeled cells in our sections belong not to CnF but to PrCnF. We found that labeled neurons in PrCnF were most numerous in the medial portion, close to the labeled neurons of the LPAG, which also contains spinal projecting neurons.

The ERS was identified and named by Paxinos and Butcher (1985), on the basis of positive acetylcholinesterase staining. In the rat and mouse, this nucleus is a group of cells in the upper hindbrain tegmentum, medial to the lateral lemniscus and dorsal to the rubrospinal tract (Paxinos and Franklin 2001). Swanson regards this nucleus as a part of the nucleus of the lateral lemniscus (Swanson 1998), but the patch of acetylcholinesterase staining distinguishes ERS from the nucleus of the lateral lemniscus and the rubrospinal tract (Paxinos and Watson 2007). In the present study, labeled neurons in this nucleus were closely allocated to the dorsal surface of the rubrospinal tract. The small number of labeled cells in ERS lies between the large population of labeled cells in PL and PTg. The labeled cells in ERS are smaller than those in the adjacent PL and PTg.

Sites of origin of descending spinal fibers not previously reported in the mouse
A projection from PL to the spinal cord has been reported in the rat (Leichnetz et al. 1978) and some other animals (Nudo and Masterton 1988). An anterograde study suggested that the MPL might project to the spinal cord in the mouse, but the data were not conclusive (Dobolyi et al. 2003). We believe that the present study is the first to convincingly demonstrate the presence of a large projection from PL to the spinal cord in the mouse. We found that intensely labeled neurons are distributed along the whole rostrocaudal extent of the
contralateral PL. It has been shown that stimulation of PL can inhibit the activity of the dorsal horn (Mokha and Iggo 1987), and these authors suggest that it may play a role in nociception.

In some species (but not in the mouse), the amygdala complex has been shown to send projections to the spinal cord (Mizuno et al. 1985; Sandrew et al. 1986; Nudo and Masterton 1988; Sanchez-Camacho et al. 2001a; Sanchez-Camacho et al. 2002). In amphibians, labeled neurons are located in the ventrocaudal telecephalon, lateral to the preoptic area (Sanchez-Camacho et al. 2001a; Sanchez-Camacho et al. 2002). In mammals, labeled neurons are located in the central and medial nuclei of the amygdala (Mizuno et al. 1985; Sandrew et al. 1986; Nudo and Masterton 1988). We found labeled neurons in the central amygdaloid nucleus (mainly in CeM) in the mouse and also in EAC and BLA. Since fibers from the amygdala do not extend below middle cervical levels (Mizuno et al. 1985), this pathway may play a role in behaviors involving head orientation.
CHAPTER III. Spinal projections from the presumptive midbrain locomotor region in the mouse
Introduction

The electrical stimulation of the mesencephalic locomotor region (MLR) evokes rhythmic stepping behaviour in decerebrate animals (Garcia-Rill et al., 1983; Skinner and Garcia-Rill, 1984; Atsuta et al., 1990; Bernau et al., 1991), indicating that this region plays a role in the generation of locomotor patterns. The MLR region is generally considered to lie deep to the superior colliculus (SC) and lateral to the periaqueductal gray (PAG) (Garcia-Rill, 1983). A number of distinct nuclear groups occupy this region, any or all of which might give rise to spinal connections that could initiate locomotor activity. The candidate nuclei in this area include the cuneiform nucleus (CnF) (Garcia-Rill et al., 1983; Skinner and Garcia-Rill, 1984; Coles et al., 1989; Allen et al., 1996), the precuneiform nucleus (PrCnF) (Liang et al., 2011), the mesencephalic reticular formation (mRt) (Garcia-Rill et al., 1985), and the peduncular tegmental nucleus (PTg) (Skinner and Garcia-Rill, 1984; Garcia-Rill et al., 1987; Coles et al., 1989). We have assumed that the position of the MLR in the mouse is homologous with the region identified in the cat, and have studied the spinal projections that arise from this region of the midbrain. In a previous retrograde tracing study in the mouse, we showed that cells in the PrCnF, the mRt, and the PTg projected to the spinal cord. We did not find labeled cells in the CnF (except for a small number of cells in its most rostral part) (Liang et al., 2011). We consider it unlikely that the PTg is an effector nucleus for the MLR because it is too deep and too caudal to be stimulated in such physiological experiments. It must be noted that the SC and the PAG have also been shown in many species to project to the spinal cord (Altman and Carpenter, 1961; Nyberg-Hansen, 1964a; Martin, 1969; Kuypers and Maisky, 1975; Graham, 1977; Harting, 1977; Castiglioni et al., 1978; Basbaum and Fields, 1979; Nudo and Masterton, 1988; Masson et al., 1991; Cowie and Holstege, 1992; Satoda et al., 2002; VanderHorst and Ulfhake, 2006; Liang et al., 2011), but neither of these areas have been implicated in the initiation of locomotion (Bernau et al., 1991). We conclude that the PrCnF and the mRt are the centers most likely to connect the MLR with the spinal cord.

The identity of the precuneiform nucleus is largely based on distinguishing it from other better known cell groups in the midbrain. In the mouse, the PrCnF extends from the level of the caudal pole of the red nucleus to the level of the rostral pole of the motor trigeminal nucleus (M5). It is located ventral to the SC, lateral to the PAG, and dorsal to the mRt (Franklin and Paxinos, 2008). Lateral to the PrCnF are a series of auditory structures: they are, from rostral to caudal: the external cortex of the inferior colliculus and the lateral lemniscus.
The caudal pole of the precuneiform nucleus lies dorsal to the cuneiform nucleus, which has distinctive horizontal band of acetylcholine esterase. The medial border of the PrCnF is formed by the distinctive boundary of the periaqueductal gray. Immunohistochemical study shows that it has scattered parvalbumin, calbindin, and calretinin positive neurons (Paxinos et al., 2009). In this study, we have examined the spinal projections of the PrCnF with retrograde and anterograde tracing methods. We have shown that cells in the PrCnF send their axons to medial areas of the lamina 7 interneuron pool in cervical and upper thoracic segments, and might therefore be an effector pathway for the MLR.

A further issue that must be considered is the definition of nuclei that lie within the presumptive MLR. There are different opinions concerning the boundaries of the CnF, PrCnF, and the mRt in different species (Panneton and Watson, 1991; Swanson, 1998; Paxinos and Watson, 2007; Franklin and Paxinos, 2008; Paxinos et al., 2009). This may explain why studies in cats and monkeys found plentiful spinal projections from the CnF (Castiglioni et al., 1978; Satoda et al., 2002), while we found very few in the mouse (Liang et al., 2011). It is probable that the size of the PrCnF in the monkey and cat has not been fully appreciated (Castiglioni et al., 1978; Satoda et al., 2002).

Materials and methods

Animals
Forty C57BL/6 mice (10 to 12 weeks old, with a weight of 25 to 30 g) were used for this study. The mice were obtained from the Animal Resource Center in Western Australia. The experimental procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales (08/48B).

Retrograde tracing
Twenty mice were used for the retrograde study. They were anaesthetised with an intraperitoneal injection of ketamine (67 mg/kg) and xylazine (10 mg/kg), before they were mounted in a mouse stereotaxic head holder (Kopf Instruments, Tujunga, CA, USA). A 5 µl Hamilton syringe (Hamilton Company, Reno, NV, USA) was mounted on a micromanipulator for spinal cord injection. The mouse adaptor was adjusted for optimal exposure of the vertebrae. Upper cervical, upper thoracic, and upper lumbar spinal cord segments were exposed by laminectomy at C2, T3 to T4, and T11 to T12, respectively. The dura on the right side was incised with the tip of a 29-gauge needle and the 5 µl Hamilton
syringe (the outer diameter of the Hamilton syringe is 0.711mm) was driven through this opening. An injection of 20 to 40 nl of Fluoro-Gold (Fluorochrome, Denver, Co, USA; diluted to 5% in distilled water) solution was made through multiple punctures into the right side of the spinal cord. The syringe was left in place for 10 minutes following the injections. Altogether, 15 mice were injected with Fluoro-gold to the upper cervical, upper thoracic, and upper lumbar segments (5 mice in each group). The control group either received normal saline injections into the spinal cord (2 mice) or Fluoro-Gold injections into the cisterna magna (3 mice). At the end of the procedure, the soft tissue and the skin were sutured and topical tetracycline (Pfizer) was sprayed over the incision. To relieve the postoperative pain, subcutaneous buprenorphine (Temgesic, Reckitt Benckiser) injections were applied.

**Anterograde tracing**
Twenty mice were anaesthetised as described above and mounted in the stereotaxic head holder. After incising the skin, the skull was penetrated with a bone drill (Fine Science Tools, North Vancouver, BC, Canada). The PrCnF was injected with 10 to 20 nl of biotinylated dextran amine (BDA) solution (10,000MW, Invitrogen, Mulgrave, VIC, Australia) (Bregma: -4.16 to -4.72 mm, midline: +1.0 to +1.25 mm, surface: -2.175 to -2.5 mm; 6 mice) using the same Hamilton syringe as for retrograde tracer injection. Control animals received the same tracer injections either into the cisterna magna (3 mice) or into the adjacent brain areas surrounding the PrCnF (BDA was deliberately injected to the adjacent PAG, SC, and mRt in 11 mice). In each case, the syringe was left in place for 10 minutes after the injection. At the end of the procedure, the soft tissue and the skin were sutured, buprenorphine was injected subcutaneously, and topical tetracycline was sprayed over the incision.

**Tissue preparation**
After survival times of 96 hours Fluoro-Gold experiments) or 6 weeks (dextran experiments), the mice were anesthetised with a lethal dose of pentobarbitone sodium (0.1 ml, 200 mg/ml) and perfused through the left ventricle with 60 ml of 0.9% normal saline containing heparin (150 IU/mouse; Sigma, Castle Hill, NSW, Australia). This was followed by 80 ml of 4% paraformaldehyde (Sigma) prepared in 0.1 M phosphate buffer (PB: Na₂HPO₄, 0.1 M; NaH₂PO₄, 0.1 M; pH = 7.4) and finally with 80 ml of 10% sucrose solution. The brain and spinal cord were removed and postfixed in 4% paraformaldehyde for two hours at 4°C, followed by cryoprotection in 30% sucrose in 0.1 M PB solution overnight at 4°C. Serial sections of the brain and spinal cord were cut at 40 µm using a Leica CM 1950 cryostat. Sections from the Fluoro-Gold injected mouse spinal cords were mounted onto gelatinized
slides and coverslipped with an anti-fade fluorescent mounting medium (Dako, Campbellfield, VIC, Australia). The tissue sections were examined with a Nikon Eclipse 80i fluorescent microscope.

**Immunohistochemistry**

The brain sections from the Fluoro-Gold injected mice were washed in 0.1 M PB and treated with 1% H$_2$O$_2$ in 50% ethanol for 30 minutes at room temperature. The sections were rinsed in 0.1 M PB and then treated with 5% goat serum in 0.1 M PB to block the non-specific sites. The sections were incubated with the primary anti- Fluoro-Gold antibody (Chemicon, 1:2000; raised in rabbit) overnight, rinsed in 0.1 M PB (3×), then treated with the secondary antibody (biotinylated goat anti-rabbit IgG; Sigma, 1:200) for 2 hours at room temperature. The sections were then washed in 0.1 M PB, and transferred to an extravidin peroxidase solution (Sigma, 1:1000) for 2 hours. After a rinse in 0.1 M PB, the sections were transferred to the 3, 3′-diaminobenzidine (DAB) reaction complex (Vector lab, Burlingame, CA, USA) until optimal color developed. At the end of the procedure, the sections were rinsed, mounted onto gelatinized slides, dehydrated in gradient ethanol, cleared in xylene, and coverslipped. The sections from the dextran injected mouse brains and spinal cords were treated with 1% H$_2$O$_2$ in 50% ethanol for 30 minutes at room temperature, followed by 5% goat serum in 0.1 M PB for 30 minutes. Then the sections were transferred to an extravidin peroxidase solution (Sigma, 1:1000) for 2 hours. The following procedures were the same as for the Fluoro-Gold immunostain.

**Data analysis**

The sections from Fluoro-Gold injected mouse brains were mapped using the Stereoinvestigator software (MicroBrightfield, Williston, VT, USA). The images were taken by an Optronics camera (Goleta, CA, USA) mounted onto a Nikon Eclipse 80i microscope, which was connected to a Dell Precision T3500 workstation. Reference to adjacent Nissl stained sections assisted in this mapping process. First, the contour was drawn at 2× magnification, and then the position of each labeled neuron was marked at 10× magnification. Cell numbers were automatically calculated by counting the marked cells in every second section through the entire precuneiform nucleus. For cell counting, 3 series of sections (prepared from 3 mice) were employed. Only labeled neurons with a visible nucleus or labeled large neurons with a clear profile were counted. The same software was applied for determining the diameters of the labeled cells (every second section across the entire precuneiform nucleus), and for taking photomicrographs of selected DAB stained sections.
The sections from dextran injected mouse brains and spinal cords were examined and photographed under the same microscope, the Nikon Eclipse 80i. The spinal cord sections were mapped onto diagrams extracted from a mouse spinal cord atlas by Watson et al. (2009), with reference to adjacent Nissl stained sections.

**Results**

**Retrograde labelling**

After cervical injections, small clusters of neurons were labeled in the PrCnF (Fig III.1a, b). In the rostralmost part of the PrCnF, there were 17.6 ± 1.6 labeled neurons per section (based on 6 sections prepared from 3 mice), situated in the proximity of the lateral periaqueductal gray (LPAG). In these sections, a few large, labeled neurons were intermingled with other labeled, oval-shaped cells of medium size (the average diameter of all labeled neurons in the PrCnF was 21.16 ± 1.67 µm × 13.89 ± 1.22 µm; mean ± S.D.;) (Fig III.1b). The number of labeled neurons tapered in more caudal sections. At the level of the rostral trochlear nucleus, their number dropped to 9.8 ± 0.8 per section, but large labeled neurons (24.12 ± 0.84 µm × 14.80 ± 0.64 µm) could still be seen. At the level caudal to the trochlear nucleus, 1 to 4 labeled neurons per section were observed at the border of the LPAG and the PrCnF. After counting every second section, there were 73.0 ± 9.2 and 24.3 ± 2.6 labeled cells in total on the ipsi- and contralateral sides, respectively (on the basis of 24 sections prepared from 3 mice).

After upper thoracic injections, there were 15.3 ± 1.5 and 6.7 ± 2.1 labeled cells in total on the ipsi- and contralateral side of the PrCnF, respectively (on the basis of 24 sections from 3 animals; Fig III.1c, d). In terms of their size (20.07 ± 5.14 µm × 13.29 ± 3.37 µm) and shape, the labeled neurons situated in the PrCnF were similar to those seen after cervical injections (Fig III.1d). After upper lumbar injections, labeled cells were not found in the PrCnF, but were still seen in the LPAG adjacent to the PrCnF (data not shown).

**Anterograde labelling**

BDA could spread to adjacent PAG, SC, and mRt after the PrCnF injection, but the ipsilateral fibers were consistently seen in our preparation. The following result was from one typical injection to the PrCnF and adjacent SC. The injection site extended from the rostral pole of the PrCnF to a level of 320 µm caudal to the rostral pole. It involved the ventralmost part of
**FigIII.1** Labeled cells in the precuneiform nucleus after spinal cord injections. **FigIII.1a** shows the spread of Fluoro-Gold in the right half of the upper cervical cord. **FigIII.1b** shows labeled cells in the precuneiform nucleus after upper cervical injections. These labeled cells are located in the medial part of this nucleus, close to labeled neurons in the lateral periaqueductal gray medially. The majority of labeled neurons in the precuneiform nucleus are on the ipsilateral side. **FigIII.1c** shows the spread of Fluoro-Gold in the right half of the upper thoracic cord. **FigIII.1d** shows labeled cells in the precuneiform nucleus after upper thoracic injections. Fewer labeled cells are seen in the medial part of this nucleus (note weakly labeled cells in the lateral part). Labeled neurons can also be seen in the adjacent mesencephalic reticular formation, lateral periaqueductal gray (with an ipsilateral predominance), contralateral oral part of the pontine reticular nucleus, paralemniscal nucleus, and bilateral superior colliculus (with a contralateral predominance). The photomicrographs in **FigIII.1b** and **FigIII.1d** demonstrate the labeled neurons in the precuneiform nucleus in the rectangular area marked on the diagram. The scale bar in the photomicrograph is 50 µm. The scale bar for the injection site is 100 µm. The scale bar for the drawings is 1mm.

the SC dorsally and the dorsalmost part of the mRt ventrally (**FigIII.2a-a’**). Labeled fibers mainly travelled along the ipsilateral ventral and lateral funiculi and entered the ipsilateral
spinal cord from the ventral horn (FigIII.2a-a”). In upper cervical segments, labeled fibers entered the spinal cord from the ventral and lateral funiculi through the lateral and medial portions of lamina 8. They traveled dorsomedially towards the central canal and were distributed in the ventral portion of lamina 7, the dorsal and central portions of lamina 8, the medial portion of lamina 9, and the ventral portion of area 10 (FigIII.2b). In mid-cervical segments, labeled fibers were distributed more medially. Most of the fibers were located in the medial and central portions of lamina 8, and the ventral portion of lamina 7. Labeled fibers were also seen in the medial part of lamina 9, where the motoneurons of axial muscles were located, and the lateral part of area 10 (FigIII.2c). As labeled fibers traveled down to the 5th and 6th cervical segments, they were more confined to the medial ventral horn. Most of them entered the spinal cord from the ventral funiculus, passing by the medial part of lamina 9, and were distributed mainly in lamina 8 and the adjacent part of medial lamina 7 (FigIII.2d). In lower cervical segments, the density of labeled fibers dropped significantly and they were mainly distributed in lamina 8, parallel with its long axis. To a lesser extent, fibers were also found in the ventromedial portion of lamina 7 and 9 (FigIII.2e). In upper thoracic segments, labeled fibers were mainly found in lamina 7 and to a lesser extent in the medial portion of lamina 8 (FigIII.2f). Labeled fibers were not found in lower thoracic and lumbar segments.

When BDA was injected to the adjacent PAG, especially the lateral part (LPAG), anterogradely labeled fibers were more numerous in upper cervical spinal cord. In the lower cervical spinal cord, labeled fibers were distributed in the medial ventral horn similar to those from the PrCnF, but fibers in the area around central canal were denser. Labeled fibers were observed in the thoracic and lumbar spinal cords bilaterally with an ipsilateral predominance. Densely packed fibers could be seen in lamina 5 (FigIII.2g). To a lesser extent, fibers were seen in area 10, laminae 7 and 8. Injections of BDA to the SC resulted in contralaterally labeled fibers in the upper cervical cord. They were similarly distributed in the spinal cord as those fibers from the PrCnF except that some fibers from the SC were also present in lamina 5 (FigIII.2h). These tectospinal fibers only terminated in the contralateral upper cervical cord and they were not seen in lower segments of the contralateral spinal cord.
Fig III. 2a a’ The injection site to the PrCnF and SC (white dash line). a” Fibers from the PrCnF travel in the ipsilateral ventral and lateral funiculi (arrows) at C3 level. The scale bar for a’ is 500 µm, the scale bar for a” is 50 µm.

Discussion

We have shown that the neurons of the mouse PrCnF project to the medial portions of laminae 7, 8, 9 and area 10 of the cervical and upper thoracic spinal cord. These projections are chiefly ipsilateral. The PrCnF is a major component of the presumptive MLR, so this projection might be a pathway by which the MLR initiates locomotor activity.

Potential overlap between projections from the PrCnF and those of the PAG and the SC in our preparation

In presenting our results, we considered the possibility that injections in the PrCnF might have labeled fibers arising in neighbouring areas, particularly the SC and the PAG. To solve this problem we studied the spinal projections from each of these areas. Our injections of retrograde tracer showed that the SC projects almost exclusively to the contralateral spinal cord, whereas the projections from the PrCnF and the PAG are mainly ipsilateral. We have concluded that the ipsilateral projections demonstrated by injection of anterograde tracer into the PrCnF are unlikely to have arisen in the SC. Moreover, the contralateral fibers labeled in the SC experiments had a different pattern of termination from those endings in the ipsilateral spinal cord. Since contralateral fibers could not be seen in segments caudal to the mid-cervical spinal cord on the contralateral side, we believe that the contralateral terminals are those arising from the SC. The projections from the PAG were ipsilateral, but the pattern of termination was very different from those arising in the PrCnF. Anterograde tracer injections...
FigIII.2b Fiber distribution in the 2nd cervical segment after the precuneiform nucleus injection of BDA. The right diagram shows labeled fibers in laminae 7 and 8, medial lamina 9, and area 10 on the ipsilateral side. The photograph on the upper left is a low-power coronal section of C2 (right is the ipsilateral side). The photograph on the lower left is a high-power coronal section of the rectangular area in the diagram on the right. The scale bar for the upper left photograph is 200 µm, the scale bar for the lower left photograph is 50 µm, the scale bar for the diagram is 100 µm.

restricted to the PAG produced a dense pattern of labeled fibers in lamina 5 and they were still seen in lumbar segments. Such a projection was not seen after the PrCnF injection.

Possible confusion regarding the identity of CnF and PrCnF in different species
There is a marked discrepancy between our findings on the spinal projection of the CnF in the mouse and the results of studies in the cat and the monkey. We found few labeled cells in the CnF after spinal injections of retrograde tracer (Liang et al., 2011), whereas Castiglioni et al. (1978) showed that the ventral portion of the monkey CnF has a substantial projection to the ipsilateral spinal cord, and Satoda et al. (2002) found that the CnF projects to the first cervical cord segment in the cat. It is possible that the neurons that we have identified in the medial portion of the PrCnF might be homologous with the medial portion of the CnF in the monkey.
and cat. Alternatively, the area recognised in the cat and monkey as the CnF might actually be an area that we would classify as PrCnF.

**FigIII.2c** Fiber distribution in the 4th cervical segment after the precuneiform nucleus injection of BDA. The right diagram shows labeled fibers in laminae 7 and 8, medial lamina 9, and area 10 on the ipsilateral side. The photograph on the upper left is a low-power coronal section of C4 (right is the ipsilateral side). The photograph on the lower left is a high-power coronal section of the rectangular area in the diagram on the right. The scale bar for the upper left photograph is 200 µm, the scale bar for the lower left photograph is 50 µm, the scale bar for the diagram is 100 µm.

**The pattern of termination of PrCnF fibers in the spinal cord**

No detailed studies have been hitherto published describing the terminal pattern of fibers originating from the MLR and projecting to the spinal cord of the mouse. However, Satoda et al. (2002) found that neurons in the area they identified as the CnF were labeled after injecting wheat germ agglutinin-horseradish peroxidase (WGA-HRP) into the central and deep regions of the ventral horn. Once again, the difference between this and our study may be based on different interpretation of the anatomy of these midbrain nuclei.

Our study showed that the PrCnF fibers terminated in the medial part of lamina 7, as well as some in laminae 8 and 9, and area 10. Larger injections involving the LPAG resulted in
labeled fibers in lamina 5. As noted above, the PAG projects strongly to ipsilateral lamina 5, especially in the thoracic and lower lumbar spinal cord. The laterocaudal SC projects to the contralateral laminae 5, 7, and 8, with relatively sparse distribution in lamina 9 (Yasui et al., 1998).

In addition, a study on the cat revealed ipsilateral terminals originating from the VLPAG in laminae 7 and 8 (Mouton and Holstege, 1994). However, the injection core also spread to the adjacent nucleus, which might have corresponded to the mouse PrCnF. In a different study on the cat and monkey, lamina 1 neurons were found to project to the caudal part of the CnF after injecting Phaseolus vulgaris leucoagglutinin (PHA-L) into lamina 1 or 1 to 3 (Craig, 1995). It is important to note that no retrogradely labeled neurons were found in lamina 1 to 3 in our study after injections of dextran into the PrCnF. This discrepancy might be explained
FigIII.2c Fiber distribution in the 8th cervical segment after the precuneiform nucleus injection of BDA. The right diagram shows labeled fibers in ventral lamina 7, medial 8, and medial lamina 9 on the ipsilateral side. The photograph on the upper left is a low-power coronal section of C8 (right is the ipsilateral side). The photograph on the lower left is a high-power coronal section of the rectangular area in the diagram on the right. The scale bar for the upper left photograph is 200 µm, the scale bar for the lower left photograph is 50 µm, the scale bar for the diagram is 100 µm.

by the more caudal location of the CnF in the mouse.

Functional significance of the PrCnF projections to the spinal cord
In our study, the PrCnF has been found to project ipsilaterally to the medial ventral horn of the cervical and upper thoracic spinal cord, suggesting that the mouse PrCnF might play a role as important in locomotion as its homologous region of the CnF in other mammals. In addition, the mRt, which is rostral to the PrCnF, has been found to have similar projections to the spinal cord. Therefore, it seems appropriate to propose that the MLR includes not only the mRt, but the PrCnF as well. The fact that the PrCnF projects only to cervical segments does not indicate a limited role in upper limb locomotion. The mechanism of how the unilateral locomotion center induces bilateral movement is unknown. One can speculate that the presumptive locomotion center in the mouse (PrCnF) sends fibers to the interneurons in the
cervical and upper thoracic segments, in turn, these interneurons activate bilateral motoneurons through the connections with the central pattern generator in the spinal cord or through the propriospinal connections since intrinsic spinal pathways (propriospinal pathways) mediate coordinated movements of the forelimb and hindlimb (Miller et al., 1998; Juvin et al., 2005; Cowley et al., 2010). The other possibility, as can be seen from my own experiment (data not shown), is that the PrCnF has a large amount of boutons terminating on neurons in the hindbrain. Some of these hindbrain areas are known to have spinal cord projections, as can be seen from the second chapter of this thesis. These

Fig III.2f Fiber distribution in the 2nd thoracic segment after the precuneiform nucleus injection of BDA. The right diagram shows labeled fibers in lamina 7, to a lesser extent in lamina 8 and 9 on the ipsilateral side. The photograph on the upper left is a low-power coronal section of T2 (right is the ipsilateral side). The photograph on the lower left is a high-power coronal section of the rectangular area in the diagram on the right. The scale bar for the upper left photograph is 200 µm, the scale bar for the lower left photograph is 50 µm, the scale bar for the diagram is 100 µm.
reticulospinal neurons might relay the signals from the PrCnF to the spinal cord neurons to trigger the bilateral movement. In the present study, we have focused on the direct connections between the PrCnF and the spinal cord, but it is also possible that initiation of locomotion might be mediated via projections to nuclei in the hindbrain, such as the gigantocellular reticular formation (Garcia-Rill and Skinner, 1987; Degtyarenko et al., 1998; Noga et al., 2003).

Most of the terminals from the PrCnF ended in laminae 7 and 8, and only a few ended among the motoneurons in lamina 9. We conclude that most of the spinal motoneurons activated by

![Image](image_url)

**FigIII.2g** Fiber distribution in the 5th lumbar segment after the periaqueuctal gray injection of BDA. The right diagram shows labeled fibers in lamina 5 (arrows) and dorsal lamina 6 (arrow) on the ipsilateral side, extending towards the central canal. The photograph on the upper left is a low-power coronal section of L5. The photograph on the lower left is a high-power coronal section of the rectangular area in the diagram on the right. The photograph on the lower right is the injection site. The scale bar for the upper left and lower right photographs is 200 µm, the scale bar for the lower left photograph is 50 µm. The scale bar for the diagram is 100 µm.
PrCnF axons must be activated via interneurons. It is well known that there is a central pattern generator for locomotion in the spinal cord, which is located in laminae 7, 8, and area 10 (Bracci et al., 1996; Cina and Hochman, 2000; Dai et al., 2005; Kjaerulff et al., 1994; Kjaerulff and Kiehn, 1996; Tresch and Kiehn, 1999). The interneurons (in lamina 7 and 8) of the central pattern generator, which are rostral to the motoneurons that directly innervate the limb muscles, also produce rhythmic activity in the limbs (Cazalets et al., 1995; Bertrand and Cazalets, 2002) and there is a capacity gradient along the rostrocaudal axis of the spinal cord.

**FigIII.2h** Fiber distribution in the contralateral 3rd cervical cord after the superior colliculus injection of BDA. The left diagram shows labeled fibers in laminae 5 (arrows), 7, 8, and 9. The photograph on the upper right is a low-power coronal section of C3 (right is the contralateral side). The photograph on the lower right is a high-power coronal section of the rectangular area in the diagram on the left. The scale bar for the upper right photograph is 200 µm, the scale bar for the lower right photograph is 50 µm, the scale bar for the diagram is 100 µm.
(Grillner and Zangger, 1979; Bracci et al., 1996; Bonnot and Morin, 1998; Bonnot et al., 2002; Christie and Whelan, 2005; Magnuson et al., 2005). Hence, there exists a possible anatomical substrate for the role of PrCnF projections to the spinal cord in the initiation of locomotion.

It is interesting to note that spinal cord neurons in lamina 1 send afferents to the CnF (Bjorkeland and Boivie, 1984; Hylden et al., 1989). Therefore, it seems reasonable to suggest that these ascending projections might form the afferent pathway of a nociceptive reflex circuit, which utilizes descending fibers originating from the PrCnF to target spinal motoneurons or interneurons to avoid noxious stimuli.
CHAPTER IV. The red nucleus and the rubrospinal projection in the mouse
Introduction

The red nucleus is a distinct neuronal cluster in all vertebrates, and it has traditionally been considered to be located in the midbrain (Wild et al., 1979; ten Donkelaar et al., 1981; Prasada Rao et al., 1987; Nudo and Masterton, 1988). However, developmental and gene expression data show that this nucleus has two origins: the caudal part belongs to the midbrain but the rostral part belongs to the diencephalon (Puelles et al., 2011). The rostral part lies caudal to the fasciculus retroflexus, and the caudal part ends just rostral to the decussation of the superior cerebellar peduncle. The oculomotor nerve traverses only in the midbrain part of the red nucleus (Fig IV.1a). Though the red nucleus is traditionally divided into the parvicellular part (RPC) and the magnocellular part (RMC), neurons of varying size are found in each part (Huber et al., 1943). Therefore the morphological division of the red nucleus does not by itself completely define the component parts of this nucleus.

Anatomical and physiological studies in species other than mice have shown that the red nucleus plays an important role in locomotion through its connections with the interneurons (Warner and Watson, 1972; Wild et al., 1979; Holstege et al., 1988; Küchler et al., 2002) and motor neurons in the spinal cord (Holstege 1987b; Holstege et al. 1988; Küchler et al., 2002), but species differences were also observed (Brown 1974; Küchler et al., 2002), indicating that rubrospinal neurons may play different roles in different species (Huber et al., 1943; Liang et al., 2011). Some spinal cord injury research on the impact of spinal cord injury focused on the degeneration, regeneration (Guizar-Sahagún et al., 2005; Harvey et al., 2005; Cao et al., 2008; Chen et al., 2008; Jefferson et al., 2011), and atrophy of the red nucleus neurons and fibers (Barron et al., 1989; 1990; Tetzlaff et al., 1991; Kobayashi et al., 1997; Ruitenberg et al., 2002; 2003; 2004). This indicates that it would be valuable to have a more detailed map of the organization of the red nucleus in rodents. We have attempted to produce such a map in the mouse, using Nissl staining, in situ hybridization, and immunofluorescence, in combination with retrograde and anterograde tracer injections. We found that large and small neurons were present in both the rostral and caudal parts of the red nucleus, and that the number and location of glutamatergic neurons matched those of the rubrospinal neurons. We conclude that rubrospinal neurons are probably all glutamatergic. Other major features of the rubrospinal neurons and their axons in the mouse were similar to those of other mammals. We believe that the present study provides a new perspective on the organization of the red nucleus in the mouse, and that the details will be of value to spinal cord injury research.
Material and methods

Animals
Thirty six C57/BL6 mice (10-12 weeks old, weight 25-30 g) were used for this study. Mice were obtained from the Animal Resource Center in Western Australia. The experimental procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales (08/48B).

Retrograde tracing
Twenty mice were used for the retrograde study. They were anaesthetised with an intraperitoneal injection of ketamine (67 mg/kg) and xylazine (10 mg/kg) before they were mounted in a mouse stereotaxic head holder (Kopf Instruments, Tujunga, CA, USA). A 5 µl Hamilton syringe (Hamilton Company, Reno, NV, USA) was mounted on a micromanipulator for spinal cord injection. The mouse adaptor was adjusted for optimal exposure of the vertebrae. Upper cervical and upper lumbar spinal cord segments were exposed by laminectomy at C2 (spinal C2-3) and T11/T12 (spinal L1-2), respectively. The dura on the right side was incised with the tip of a 29-gauge needle and the 5 µl Hamilton syringe (the outer diameter of the Hamilton syringe is 0.711 mm) was driven through this opening. An injection of 20 to 40 nl of fluoro-gold (Fluorochrome, Denver, Co, USA; diluted to 5% in distilled water) solution was made through multiple punctures into the right side of the spinal cord. The syringe was left in place for 10 minutes following the injections. Altogether, 16 mice were injected with fluoro-gold to the upper cervical and upper lumbar segments (8 mice in each group). The control group either received normal saline injections into the spinal cord (2 mice) or fluoro-gold injections into the cisterna magna (2 mice). At the end of the procedure, the soft tissue and the skin were sutured and topical tetracycline (Pfizer) was sprayed over the incision. To relieve the postoperative pain, subcutaneous buprenorphine (Temgesic, Reckitt Benckiser) injections were applied.

Anterograde tracing
Sixteen mice were anaesthetised with an intraperitoneal injection of ketamine (67 mg/kg) and xylazine (10 mg/kg), and then they were mounted in a mouse stereotaxic head holder (Kopf Instruments, Tujunga, CA, USA). A 5 µl Hamilton syringe (Hamilton Company, Reno, NV, USA) (same as retrograde study) was mounted on a micromanipulator for tracer injection. After incising the skin, the skull was drilled with a bone drill (Fine Science Tools, North...
10-20 nl biotinylated dextran amine solution (Invitrogen) was injected into the red nucleus (Bregma: -3.16-4.16 mm, midline: +0.5~1.0 mm, surface: -3.75~4.25 mm; 8 mice). Control animals received the same tracer injections either into the cisterna magna (2 mice) or into the adjacent brain areas surrounding the red nucleus (5 mice). In each case the syringe was left in place for 10 minutes after the injection. At the end of the procedure, the soft tissue and the skin were sutured, Temgesic was injected subcutaneously, and tetracycline was sprayed over the incision.

**Tissue preparation**

After a period of 7 days and 6 weeks survival time for the FG and BDA injections respectively, mice were anesthetised with a lethal dose of pentobarbitone sodium (0.1 ml, 60 mg/ml) and perfused through the left ventricle with 60 ml of 0.9% normal saline containing heparin (150 IU/mouse; Sigma), followed by 80 ml of 4% paraformaldehyde (Sigma) prepared in 0.1 M phosphate buffer (PB: Na2HPO4, 0.1 M; NaH2PO4, 0.1 M; pH = 7.4) and finally with 80 ml of 10% sucrose solution. The brain and spinal cord were removed and postfixed in 4% paraformaldehyde for two hours at 4°C, followed by cryoprotection in 30% sucrose in 0.1 M PB solution overnight at 4°C. 40 µm thick serial coronal sections were cut from the brain and spinal cord using a Leica cryostat CM 1950. Every third sections from FG injected mice were mounted directly to slides and coverslipped with anti-fade fluorescent mounting medium (Dako). The other two series of sections were stained by Nissl and neurofilament H non-phosphorylated (SMI-32) antibody respectively. Sections from BDA injected mice were washed in 0.1 M PB, and reacted with HRP conjugated extravidin (Sigma) for 2h at room temperature after deactivating the endogenous peroxidise with 1% H2O2. After a rinse in 0.1 M PB, the sections were transferred to the 3,3’-Diaminobenzidine (DAB) reaction complex (Vector lab, Burlingame, CA, USA) till optimal colour developed. Sections were rinsed, mounted onto gelatinized slides, dehydrated in gradient ethanol, cleared in xylene, and coverslipped. Some sections were counterstained with Nissl stain.

**Immunofluorescence**

The sections from 6 FG injected mouse brains (3 from cervical injections of FG, 3 from lumbar injections of FG) were incubated in the primary neurofilament H non-phosphorylated (SMI-32) monoclonal antibody (Covance, 1:1000; raised in mouse) (SMI-32 is a non-phosphorylated neurofilament, a cytoskeletal marker for neurons. It has been reported that it is expressed in some cranial nuclei and precerebellar neurons in the vestibular nucleus) and
sections from 3 BDA injected mouse spinal cord were incubated in anti-calretinin polyclonal antibody (Chemicon, 1:2000; raised in rabbit) overnight after washing in 0.1 M PB. The mouse brain sections were then treated with the Alexa fluor 594 conjugated goat anti- mouse IgG (Invitrogen, 1:200), mouse spinal cord sections were treated with Alexa fluor 594 conjugated goat anti- rabbit IgG (Invitrogen, 1:200) and Alexa fluor 488 conjugated avidin (Invitrogen, 1:200) for 2 hours at room temperature. At the end of the procedure, the sections were rinsed, mounted onto slides, and coverslipped with anti-fade fluorescent mounting medium (Dako).

Data analysis
After fluoro-gold injections to the spinal cord and BDA injections to the red nucleus, brain (including SMI-32 antibody stained sections) and spinal cord sections were examined with a Nikon Eclipse 80i microscope, with reference to a mouse brain atlas (Franklin and Paxinos, 2008) and a spinal cord atlas (Watson et al, 2009). Photomicrographs (including DAB stained sections and fluorescent sections) were taken with the same microscope. FG and SMI-32 antibody labeled neurons in the red nucleus were counted in every third section across the entire red nucleus using the Stereoinvestigator software (MicroBrightfield, Williston, VT, USA). In situ hybridization data for the complement component 1, q subcomponent-like 2 gene (C1QL2), vesicular glutamate transporter 2 gene (vGluT2), glutamic acid decarboxylase gene (GAD67) were obtained from the Allen Brain Atlas website (http://mouse.brain-map.org). Positive neurons were counted in every fourth sections for C1QL2 gene and vGluT2 gene, and every eighth sections for GAD67 (section thickness is 25 µm). Labeled neurons with a visible nucleus or with an 'empty' nuclear profile were counted. Nuclear diameter was measured with the Stereoinvestigator software. The counts of rubrospinal neurons, and SMI-32, C1QL2, vGluT2, and GAD67 positive neurons were corrected with the Abercrombie formula (1946). In this formula, the corrected count (A) is calculated by multiplying the total number of neurons counted (P) by a factor in which the section thickness is divided by the section thickness plus the diameter of the neuronal nucleus.

Results
Neuronal populations in the red nucleus
The red nucleus in the mouse extends from the caudal midbrain to the rostral boundary of the first diencephalic prosomere (p1), just caudal to the fasciculus retroflexus (fr). The part which lies in the midbrain is predominantly magnocellular, whereas the rostral (diencephalic) part
contains both small and large neurons according to Nissl stained sections (FigIV.1a-c). The

**FigIV.1 a.** A diagram showing the location of the red nucleus in a horizontal section of the mouse brain. The diencephalic (p1) part is caudal to the fasciculus retroflexus and rostral to the oculomotor nerve fibers. The midbrain (m1) part is traversed by the oculomotor nerve fibers and rostral to the decussation of the superior cerebellar peduncle. p1: prosomere 1, m1: mesomere 1. The thick dashed line indicates the contour of the red nucleus. The thin dashed line indicates the probable location of the boundary between the p1 and m1. **b.** A Nissl-stained coronal section through the rostral part of the red nucleus. Large (star) and small (arrow) neurons are seen in the red nucleus, but the majority are small neurons. **c.** A Nissl-stained coronal section through the caudal part of the red nucleus. Large (star) and small (arrow) neurons are seen in the red nucleus at this level, but the majority are large neurons. con: contralateral, ip: ipsilateral, ml: medial lemniscus, mlf: medial longitudinal fasciculus. The scale bar is 50µm.

midbrain part of the nucleus is traversed by the emerging fibers of the oculomotor nerve. We have attempted to further characterize the neuronal populations of the red nucleus in terms of their descending connections, Nissl histology, immunofluorescence, and gene expression.

FG injections into both the upper cervical (FigIV.2a) and lumbar cord (FigIV.2i) revealed labeled neurons in both the rostral and caudal parts of the red nucleus. The majority of labeled neurons in the rostral part were located in the ventrolateral portion of the contralateral red nucleus (FigIV.2b-d, j-l). Labeled neurons were more medially placed in more caudal sections (FigIV.2e-h, m-p). A topographic organization of these rubrospinal neurons was observed. Neurons projecting to the lumbar cord were more ventrolaterally located than those projecting to the cervical cord. The majority of the rubrospinal neurons are medium sized neurons (21.3±1.4µm×16.9±2.6 µm), however, large (30.2±4.6µm×21.2±4.9 µm) and small (16.7±1.7 µm×13.6±1.6 µm) rubrospinal neurons are also seen. Most of the large neurons are clustered in the middle session of the red nucleus in coronal sections with some small rubrospinal neurons surrounding them. After counting every third section, the estimated number of labeled neurons on the contralateral side (the total number of labeled neurons) after cervical injections was 2117.3±154.8 and these neurons were approximately two thirds
(66.2±9.2%) of the total neurons (3200.9±230.8, calculated from Nissl stained sections) in
the red nucleus. Lumbar injections of FG resulted in 789.4±78.4 labeled neurons —
approximately one quarter (28.0±4.1%) of the total number of neurons. By calculating the
percentage of labeled neurons in each section (every third section) against the total number of
labeled neurons (total number of every third sections), it could be seen that sections in the

middle part of the red nucleus had the largest number of labeled neurons. Numbers of labeled
neurons tapered in the rostral and caudal parts of the red nucleus (FigIV.2q-s).

Double staining with SMI-32 antibody showed that 59.9±11.1% of the SMI-32 positive
neurons (total number was 441.9±99.6) were cervical cord projecting neurons and 24.4±2.24%
of SMI-32 positive neurons were lumbar cord projecting neurons. The most double labeled
neurons were located in the ventromedial part of the red nucleus, and fewer neurons were

FigIV.2 Labeled neurons in the red nucleus after spinal cord injections. a. An injection site in
the upper cervical cord. b-h. Labeled neurons in the red nucleus after the injection shown in a.
Labeled neurons are placed more medially in more caudal sections in the red nucleus. a’-g’
are adjacent Nissl sections caudal to FG stained sections i. An injection site in the upper
lumbar cord. j-p. Labeled neurons in the red nucleus after the injection shown in i. j’-o’ are
adjacent Nissl sections caudal to FG stained sections. The distribution of labeled neurons is
similar to that seen after cervical injections, but their position is more ventral and lateral. q
and r. The percentage of labeled neurons in each section (every third section) compared to
the total number of labeled neurons in every third sections in the red nucleus after cervical
and lumbar injections respectively. The largest proportion of labeled neurons is in the middle
sections of the red nucleus. The distribution of rubrospinal neurons rostrocaudally is similar.
s. A diagram showing the distribution of labeled neurons (black dots) in each section. D:
dorsal, L: lateral. The scale bar for injection sites is 100 µm. The scale bar for photographs of
the red nucleus is 50 µm.
located in the lateral part of the rostral half

*Fig IV. 2* continued.

of the red nucleus (*Fig IV.3a-c; Fig IV.4a-c*). However, the overall percentage of double labeled neurons was higher in the caudal part of the red nucleus than in the rostral part (*Fig IV.3d-f; Fig IV. 4d-f*).

*In situ* hybridization against the mRNA of complement component 1, q subcomponent-like 2 gene (C1QL2), a landmark for red nucleus neurons, shows that C1QL2 positive neurons are mainly ventrolaterally distributed in the rostral part and ventromedially distributed in the caudal part of the red nucleus (data from Allen Brain Atlas- [http://mouse.brain-map.org](http://mouse.brain-map.org)) (*Fig IV.5a-c*). This suggests that the majority of C1QL2 positive neurons are located in the conventionally recognized magnocellular part of the red nucleus with a total number of
2244.2±187.2, which is close to the number of cervical projecting red nucleus neurons.

However, in situ hybridization against the mRNA of glutamic acid decarboxylase 1 gene (GAD67) and solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6 gene (Slc17a6, also known as vesicular glutamate transporter 2-vGluT2) (data from Allen Brain Atlas- http://mouse.brain-map.org) shows that the caudal part of the red nucleus is positive only for vGluT2 gene, whereas the rostral part is positive for both vGluT2 and GAD67 genes (Fig IV.6a-d). By counting the neuron numbers related to vGluT2 and GAD67 expression, we find that 2181.7±12.1 neurons are positive for vGluT2 and their location is very similar to that of the rubrospinal neurons. We find that 2353.7±65.5 neurons are positive for GAD67. The total of vGluT2 and GAD67 positive neurons is approximately 4500, which is greater than the total number of neurons counted in Nissl stained sections.
However, it has been shown that GABA and glutamate can coexist in adult mouse neocortex neurons (Hill et al., 2000). Therefore, the higher number may be explained by the possibility that neurons expressing both GABA and glutamate are common in the rostral part of the red nucleus.

**FigIV.3** Double labelling with SMI-32 antibody in FG stained sections after cervical injections. a-b. A rostral section stained with FG (a) and SMI-32 antibody (b). Double-labeled neurons (arrows) are 50.0% of SMI-32 positive neurons. c. Merged photo of FG and SMI-32 antibody staining. d-e. A caudal section stained with FG (d) and SMI-32 antibody (e). Double labeled neurons (arrows) are 80.0% of SMI-32 positive neurons. f. Merged photo of FG and SMI-32 antibody staining. Note the oculomotor nerve fibers (star) traversing in the red nucleus. D: dorsal, L: lateral. Scale bar is 50 µm.

**Anterograde labelling**

Injections of BDA into the rostral red nucleus (**FigIV.7a**) showed that labeled fibers crossed the midline at the level of the ventral tegmental decussation and then travelled caudally. In the rostral hindbrain, labeled fibers were located medial to the intermediate nucleus of the lateral lemniscus (**FigIV.7b**) and the motor root of the trigeminal nerve. At the level of the motor trigeminal nucleus (5N), fibers went through between the motor trigeminal nucleus and the principal sensory trigeminal nucleus (Pr5) and then became applied to the ventrolateral surface of the hindbrain, ventromedial to the facial nerve (7n) (**FigIV.7c**). At the level of the facial nucleus (7N), fibers moved to a location medial to the spinal trigeminal nerve (sp5) and ventrolateral to the facial nucleus. These fibers maintained this location in the rest of the
hindbrain.

In the spinal cord, the rubrospinal tract was seen in the dorsolateral part of the lateral funiculus (FigIV.7d, e, g, h) in the entire spinal cord on the contralateral side, ventral to the lateral spinal nucleus. The rubrospinal fibers were located between the lateral spinal nucleus and the calretinin positive fibers in the lateral funiculus (FigIV.7f). This tract formed a wedge shape with the tip going towards the gray matter. In the gray matter of the spinal cord, densely labeled fibers were mainly seen in laminae 5, 6, and the dorsal portion of lamina 7 (FigIV.7d, g, h, l). Fewer fibers were found in lamina 8 and 10 after rostral red nucleus injections. Some fibers crossed the area 10 and extended to the other side (FigIV.7m). On the ipsilateral side, the rubrospinal tract could also be seen, but with fewer fibers, and projections of these fibers to the gray matter were very sparse in laminae 5, 6, and dorsal part of lamina 7 (FigIV.7i and j). Ipsilateral fibers were not seen in the lumbar cord and lower segments. Since the injection site in one case also involved a portion of the ventral tegmental decussation, it is possible that these ipsilateral fibers might not originate from the ipsilateral

![FigIV.4](image)

Double labelling with SMI-32 antibody in FG stained sections after lumbar injections. **a-b.** A rostral section stained with FG (a) and SMI-32 antibody (b). Double labeled neurons (arrows) are 15.0% of SMI-32 positive neurons. **c.** Merged photo of FG and SMI-32 antibody staining. **d-e.** A caudal section stained with FG (d) and SMI-32 antibody (e). Double labeled neurons (arrows) are 50.0% of SMI-32 positive neurons. **f.** Merged photo of FG and SMI-32 antibody staining. D: dorsal, L: lateral. Scale bar is 50 µm.
red nucleus, but from the red nucleus contralateral to the injection site. An injection of BDA into the rubrospinal tract below the decussation (data not shown) confirmed that fibers of passage could be labeled with BDA.

**FigIV.5** Images of sections of in situ hybridization preparations to show expression of C1QL2 gene from Allen Brain Atlas website (http://mouse.brain-map.org). **a.** A rostral section of the red nucleus. C1QL2 positive neurons are located in the lateral portion of the red nucleus. **b.** A middle section of the red nucleus. C1QL2 positive neurons are located in the ventral and dorsomedial portions of the red nucleus. **c.** A caudal section of the red nucleus. C1QL2 positive neurons are located in the medial portion of the red nucleus. The scale bar is 211 µm. We are indebted to the creators of Internet-based collections of brain anatomy whose sections we have used in the preparation of the present paper. It is the Allen Institute for Brain Science (Lein et al., 2007 and the Allen Brain Atlas [Internet], 2008 — http://mouse.brain-map.org/welcome.do).

Injections of dextran into the nuclei that lie dorsal to the red nucleus, including the periaqueductal gray (PAG), interstitial nucleus of Cajal (InC), nucleus of Darkschewitsch (Dk), and the mesencephalic reticular nucleus (mRt) resulted in distinctly different distribution patterns. Projections from these nuclei mainly travelled in the ipsilateral ventromedial funiculus and spread mainly in lamina 7, 8, and 9. Their fibers also extended towards the dorsal horn, and could therefore be differentiated from the terminating pattern of rubrospinal fibers (**FigIV.7m**).

Injections to the middle part of the red nucleus resulted in densely labeled fibers in the ventral part of lamina 5, lamina 6, and 7 in the cervical cord. In C8 and T1 segments, some fibers were seen in the dorsolateral hand motor neuron group (see Watson et al., 2009). The thoracic and lumbar segments had fewer labeled fibers in laminae 5, 6, and 7. When dextran was injected to the dorsal part of the caudal red nucleus, more densely labeled fibers were found in lamina 5 in the lumbar cord (**FigIV.7k, l**). In the latter case, the injection site spread to the lateral periaqueductal gray, and labeled fibers were mainly seen in lamina 5 of the ipsilateral spinal cord (data not shown).
**FigIV.6** Images of sections of *in situ* hybridization preparations to show expression of vGluT2 gene and GAD67 gene from Allen Brain Atlas website (http://mouse.brain-map.org).

a. A section shows that vGluT2 positive neurons are present in the prosomere 1 part of the red nucleus (p1R).

b. A section adjacent to a shows that GAD67 positive neurons are also present in p1R.

c. A caudal section shows that GAD67 positive neurons are absent in the mesomere 1 part of the red nucleus (m1R), but present in the pararubral nucleus (PaR).

d. A section adjacent to c shows that vGluT2 positive neurons are present in the m1R. Dk: nucleus of Darkschewitsch, 3N: oculomotor nucleus, IP: interpeduncular nucleus, mlf: medial longitudinal fasciculus. The scale bar is 314 µm. We are indebted to the creators of Internet-based collections of brain anatomy whose sections we have used in the preparation of the present paper. It is the Allen Institute for Brain Science (Lein et al., 2007 and the Allen Brain Atlas [Internet], 2008 — http://mouse.brain-map.org/welcome.do).

When the caudal most part of the red nucleus was injected (**FigIV.7n**), densely labeled fibers
were seen in the laminae 5, 6, and 7 of lumbar and sacral segments (FigIV.7q and r). A few fibers were also observed in lamina 7 in segments of the thoracic cord. In T1 and L5-L6, a small number of fibers was seen in the dorsolateral motor neuron group (FigIV.7o-r).

Discussion

Subdivisions of the red nucleus
We have studied the organization of the mouse red nucleus with a variety of techniques. We have found that the red nucleus is not a homogeneous neuron group; it contains a number of discrete populations of neurons. Rubrospinal neurons occupy the ventrolateral portion of the rostral part of the red nucleus and the dorsomedial portion of the caudal part of this nucleus. vGluT2 gene and C1QL2 gene positive neurons are present in the same regions as rubrospinal neurons. The distribution of SMI-32 positive neurons is more limited: they are mainly located among rubrospinal neurons in the ventromedial part of the red nucleus; approximately 60% of SMI-32 positive neurons are double labeled by FG after cervical injections of FG, 24% are double labeled by FG after lumbar injections of FG.

It has been customary to divide the red nucleus into a rostral parvicellular part and a caudal magnocellular part (Huber et al., 1943). However, gene expression data on the development of the red nucleus show that the rostral part of the red nucleus belongs to prosomere 1 of the diencephalon, whereas the caudal part of the red nucleus, traversed by the oculomotor nerve, belongs to the midbrain (Puelles et al., 2011).

Our retrograde tracing experiments show that rubrospinal neurons are found in both the rostral and the caudal parts of the red nucleus, and there is no clear boundary to separate these two spinal projecting neuron groups. In fact, there were actually more rubrospinal neurons in the rostral diencephalic part than in the caudal midbrain part of the red nucleus (see Fig. 2Q-S). However, the correlation of retrograde labelling and in situ hybridization data shows that the rubrospinal neurons are very likely to be vGluT2 positive. Surprisingly, the rubrospinal neurons located in the area that lacks GAD67 expression constitute approximately 36% of the total rubrospinal neurons, and those in the GAD67 expression area constitute 64%. Note that the area lacking GAD67 expression contains the minority of the vGluT2 expressing neurons, and represents the midbrain (m1) part of the nucleus. No sharp boundary is observed between the diencephalic and midbrain parts of the red nucleus, but it must be pointed out that the
Fig IV.7 Labeled rubrospinal fibers in the spinal cord after BDA injections to the red nucleus. 

a. Injection site in the rostral red nucleus which involved the interstitial nucleus of Cajal (star). b. Crossed rubrospinal fibers (arrow) in the rostral hindbrain, medial to the intermediate nucleus of the lateral lemniscus (arrow). c. Rubrospinal fibers in the hindbrain, medial to the facial nerve. d. Rubrospinal fibers in the contralateral C3 (arrow). Note the ipsilateral fibers, which arise from the interstitial nucleus of Cajal. e. Rubrospinal fibers in the contralateral C7. The rubrospinal fibers are present in laminae 5, 6, and 7 (arrows). f. Alexa fluor 488 labeled rubrospinal fibers (arrows) and Alexa fluor 594 labeled calretinin fibers (stars) and neurons in the contralateral C4. Note some rubrospinal fibers mix with calretinin positive fibers (lower arrow). g. Labeled rubrospinal fibers in the contralateral T5. Fibers are present in lamina 5, dorsal lamina 7, and the intercalated nucleus (arrows). Note some fibers cross the midline and extend into the ipsilateral side. Ipsilateral fibers from the interstitial nucleus of Cajal are present in laminae 5, 7, and 8. h. Dense fibers in lamina 5 in L4 (arrow). i. Injection site to the medial part of the caudal red nucleus (arrow). Note the crossing fibers ventral to the injection site. j. Ipsilateral rubrospinal fibers in laminae 5 and 6 in C4 (arrows). Some fibers in laminae 7 and 8 are from the interstitial nucleus of Cajal. k. Injection site in the dorsal portion of the caudal red nucleus (star). l. Densely labeled rubrospinal fibers in laminae 5 and 6 (arrow) in the contralateral L5. Note some fibers in the dorsolateral motor neuron group. m. Labeled fibers from the interstitial nucleus of Cajal. Fibers are present in laminae 5, 6, 7, and 8 (arrows). Note the fibers from the contralateral side. n. Injection site in the caudal most part of the red nucleus (star). o. Labeled rubrospinal fibers in the contralateral T1. p. Higher magnification of fibers in the dorsolateral motor neuron group in the rectangular area in o. Note the boutons on two possible motor neurons. q. Labeled rubrospinal fibers in the contralateral L6. r. Higher magnification of the rubrospinal fibers in the rectangular area in q. Note the dense fibers in laminae 6 and 7, boutons on two possible motor neurons (arrows). 7n: facial nerve, fr: fasciculus retroflexus, PAG: periaqueductal gray, Pn: pontine nucleus, Pr5: the principal sensory trigeminal nucleus, rs: rubrospinal tract. The scale bar for a-d, i, k, n, o, q is 200 µm. The scale bar for e-h, j, l, m, p and r is 50 µm.
Fig IV.7 continued.

The present study provides evidence for the segmentation of the red nucleus.

It looks confusing that GABA and glutamate can coexist in one neuron. In fact, there is a report of the coexistence of these two types of neurotransmitters in the mouse neocortex (Hill et al. 2000). It is highly possible that these two types of neurotransmitters are also coexistent in the mouse red nucleus. But it could not be excluded that the stereological counting of red nucleus neurons was not precise because one section out of four or eight sections for GABA and glutamate respectively were counted. Though the other parameters are known: the section thickness is 25µm and the nuclear diameter can be borrowed from Nissl stained sections. It will be resolved in the future by double labelling of the rubrospinal neurons.

The red nucleus has been known to receive cerebral projections from the motor and premotor areas and these projections are topographically organized (Kuypers and Lawrence 1967; Monakow et al., 1979; Tokuno et al., 1995). The magnocellular part of the red nucleus mainly receives afferents from the precentral gyrus, whereas the parvicellular part primarily receives afferents from the precentral gyrus, the adjacent frontal area rostral to the precentral gyrus, and the supplementary motor area. Given the somatotopical arrangement of the rubrospinal neurons, the cortico-rubro-spinal projection might also be topographically organized but this has not been investigated in this thesis.
Double staining with SMI-32 antibody in FG stained sections after spinal cord injections shows that 60% of SMI-32 positive neurons are cervical cord projecting neurons, and the majority of them are in the medial portion of the caudal 2/3 of the red nucleus. Therefore, SMI-32 serves as a useful signal for rubrospinal neurons, especially for those in the caudal part. In a primate spinal cord injury study, SMI-32 antibody was used as a marker to label rubrospinal neurons (Wannier-Morino et al., 2008). After unilateral section of the spinal cord, this study showed that the number of SMI-32 positive neurons was decreased. It is interesting to note that the total number of SMI-32 positive neurons in this monkey study ranged from 39 to 174. In contrast, the mouse has more than 400 SMI-32 positive neurons, and 60% of these neurons project to the cervical cord. Considering the fact that the mouse is a very small mammal, this is consistent with a view that the rubrospinal projection is considerably more important for limb movement in the mouse than in the monkey.

C1QL2 expression is also a useful marker for mouse red nucleus neurons, and the number of C1QL2 positive neurons is similar to that of FG labeled neurons. However, their location, compared to the results of retrograde labelling, suggests that some of them are not spinal cord projecting neurons, especially in the rostral part of the red nucleus. This is further evidence that the red nucleus contains overlapping populations of different neuron types.

The rubrospinal tract
The mouse rubrospinal tract crosses the midline in the ventral tegmental decussation and travels in the ventrolateral hindbrain. In the spinal cord this tract occupies a dorsolateral position, taking a wedge shape. In the gray matter of the spinal cord, fibers are mainly distributed in laminae 5, 6, and the dorsal part of lamina 7. Some fibers are also observed in the dorsolateral motor neuron groups in C8-T1 and L5-L6. An ipsilateral rubrospinal tract is also present but with fewer fibers terminating in laminae 5, 6, and the dorsal part of lamina 7. Our result is consistent with previous studies on other species (rat: Brown, 1974; Antal et al., 1992; Yasui et al., 2001; Küchler et al., 2002; pigeon: Wild et al., 1979; cat: Gibson et al., 1984; McCurdy et al., 1987; Holstege 1987b; Fujito and Aoki, 1995; tree shrew: Murray et al., 1976; monkey: Shapovalov et al., 1971; Ralston et al., 1988; marsupial-possum: Warner and Watson, 1972).

The present study has demonstrated that the dorsolateral motor neuron group in the C8-T1 is
innervated by the rubrospinal fibers as reported in some other studies (Shapovalov et al., 1971; Holstege 1988; McCurdy et al., 1987; Ralston et al., 1988; Fujito and Aoki, 1995; Küchler et al., 2002). The other finding is that the dorsolateral motor neuron group at L5-L6 receives rubrospinal projections. This group supplies the foot muscles (Watson et al., 2009). This latter observation has previously been reported in physiological studies (Hongo et al., 1969; Shapovalov et al., 1971) and in one anatomical study on the monkey (Holstege et al., 1988). However, there is no evidence from electrophysiology that the rubrospinal fibers have monosynaptic projections to the spinal cord motoneurons. The present study is the first one to show the anatomical evidence of probable monosynaptic connections between the red nucleus and distal limb motor neuron groups in the mouse. This suggests the important role of the red nucleus in the distal limb movement.

Ipsilateral rubrospinal tract and fibers were observed in the present study, but they are few in number. Our retrograde tracing studies showed that labeled neurons are present in the ipsilateral red nucleus. This is similar to findings in the rat (Antal et al., 1992), cat (Holstege 1987), and a marsupial (Warner and Watson, 1972). In the cat, ipsilateral fibers were shown to terminate only in the cervical and upper thoracic cord (Holstege, 1987b). In the present study, a few ipsilateral fibers were seen in the cervical and thoracic cord in one case. However, in this case the injection site spread into the ventral tegmental decussation, and it is possible that these fibers of passage might have been labeled by the injection. In a separate experiment in which the injection site was centred on the rubrospinal tract caudal to the decussation, labeled fibers were found in the ipsilateral spinal cord, showing that BDA can be taken up by fibers of passage. A further possibility is that rubrospinal fibers that cross the midline in the spinal cord might have been labeled in retrograde tracing experiments (Liang et al, 2011), and this label might be transmitted to neurons in the ipsilateral red nucleus (after recrossing in the ventral tegmental decussation).

In the mouse spinal cord, calretinin antibody staining reveals a negative area between the calretinin positive fibers in the dorsolateral funiculus which corresponds with the location of the rubrospinal tract (Watson et al., 2009). We found that the rubrospinal tract is located between the calretinin positive fibers in the dorsolateral funiculus, with a small number of rubrospinal fibers intermingled with the calretinin positive fibers. Therefore, calretinin antibody staining may serve as a useful landmark for studies that focus on the degeneration and regeneration of rubrospinal fibers after spinal cord injury.
CHAPTER V. General discussion
The present thesis aims to map all the spinal projecting neuronal groups in the mouse through retrograde tracer injections. In the first set of studies, all the spinal projecting neuronal groups were mapped. Then, the axonal terminals of the precuneiform nucleus in the spinal cord were investigated with retrograde and anterograde tracer injections. Finally, the organization of the red nucleus and the rubrospinal fibers in the spinal cord were studied with retrograde and anterograde tracer injections, and immunofluorescence. By comparing my data with in situ hybridization data from Allen Brain Atlas website, the neurotransmitter used by the rubrospinal neurons and some landmarks were suggested.

5.1 Results summary

The present thesis has focused on brain projections to the spinal cord in the mouse. Besides the widely studied spinal projecting neuronal groups, two nuclei which have not been reported to project to the spinal cord were identified. Furthermore, two nuclei – the precuneiform nucleus and the red nucleus have been well investigated for the location of spinal projecting neurons and the distribution of their axonal terminations in the spinal cord.

Chapter II maps the spinal cord projecting neuronal groups in the mouse. The majority of the spinal cord projecting neuronal groups are in similar locations as those in other species. Two additional nuclei, the precuneiform and the epirubrospinal, were found to project to the spinal cord in the mouse. These results have not been reported before in any species. Another two areas, the amygdala and the paralemniscal nucleus, which have been known in some species to project to the spinal cord, were also found to do so in the mouse.

In chapter III, the axonal terminal distribution of the precuneiform nucleus was studied using an anterograde tracer. Firstly, in the retrograde study, labeled neurons were only found in the medial part of the precuneiform nucleus after cervical and upper thoracic injections. Secondly, in the anterograde study, biotinylated dextran was injected to the precuneiform nucleus and other surrounding nuclei. Labeled axonal terminals from the precuneiform nucleus in the spinal cord were mainly found in lamina 7 and, to a lesser extent, in laminae 8, 9, and 10 of the spinal cord. These fibers are different to those from the superior colliculus. Axons from the superior colliculus cross the midline in the caudal midbrain and terminate in laminae 5, 7, 8, and 9 in the contralateral spinal cord. These axons end in the upper cervical cord. Axons from the adjacent lateral periaqueductal gray differ from those originating in the precuneiform nucleus. They are bilaterally distributed with an ipsilateral predominance. The
majority of these axons terminate in laminae 5, 6, and 7. These axons were still seen in the lumbar cord.

In chapter IV, the organization of the red nucleus and the rubrospinal projection were investigated with a range of techniques (Nissl stain, immunofluorescence, retrograde tracer injections into the spinal cord, anterograde tracer injections into the red nucleus, and in situ hybridization). In the retrograde study, labeled neurons were found in both the rostral (diencephalic part) and the caudal (midbrain part) parts of the contralateral red nucleus after cervical and lumbar cord injections of fluoro-gold. These neurons are located in the ventrolateral part of the red nucleus in the rostral part and are placed medially in more caudal sections. Topographic organization of these labeled neurons was observed as labeled neurons projecting to the lumbar cord are more ventrally and laterally positioned than those projecting to the cervical cord. After counting every third section of the entire red nucleus, about two thirds of the total neurons project to the cervical cord and about one quarter to the lumbar cord (there are potential overlaps) (total number is 3200.96±230.80). Immunofluorescence staining with SMI-32 antibody showed that approximately 60% of SMI-32 positive neurons are cervical-cord projecting neurons, and 24% are lumbar-cord projecting neurons. SMI-32 positive neurons are mainly located in the medial portion of the caudal part of the red nucleus. Comparison of the distribution of rubrospinal neurons in the present thesis with in situ hybridization against vGluT2 mRNA (from Allen Brain Atlas website – http://mouse.brain-map.org) shows that the number and location of glutamatergic neurons match those of rubrospinal neurons. In situ hybridization against the complement component 1, q subcomponent-like 2 gene (C1QL2) (from Allen Brain Atlas website – http://mouse.brain-map.org) shows that many C1QL2 positive neurons might be rubrospinal neurons, especially in the caudal part of the red nucleus. In the anterograde tracing experiment performed herein, rubrospinal fibers were seen to cross the midline at the level of the ventral tegmental decussation and form a compact bundle medially to the intermediate nucleus of the lateral lemniscus. More caudally, the tract lies ventromedial to the spinal trigeminal tract. In the spinal cord, these fibers travel in the dorsal portion of the lateral funiculus, between the lateral spinal nucleus and the calretinin positive fibers of the lateral funiculus. Rubrospinal fibers terminate in contralateral laminae 5, 6, and the dorsal part of the lamina 7 at all spinal cord levels. A few fibers were seen next to neurons in the dorsolateral part of lamina 9 at levels of C8-T1 (hand motor neurons) and L5-L6 (foot motor neurons), consistent with a view that rubrospinal fibers may play a role in distal limb movement in rodents.
5.2. Neuronal groups in the prosencephalon

5.2.1. The cerebral cortex
In the present thesis, the cerebral cortex of the mouse issues spinal projections from its motor and somatosensory areas including the primary and secondary areas. Though the density of labeled neurons in motor cortical areas is higher than that of somatosensory cortical areas, labeled neurons in the somatosensory areas are more widely distributed than in the motor areas. These results are similar to those of other previous retrograde studies in the mouse (Sbriccoli et al., 1995; Tsukamoto et al., 2003). Consistent with the result of the present thesis, Bareyre et al (1995), using a special marker for the corticospinal neurons in the mouse – Thy-1 YFP, demonstrated the complete labelling pattern of these spinal projecting neurons. In rats, cats and monkeys, different nomenclature is applied to identify cortical areas, but the labelling of spinal projecting neurons is similar to that of the mouse (in rats: Schwanzel-Fukuda et al., 1984; Miller 1987; Masson et al., 1991; in cats: Armand and Aurenty, 1977; Groos et al., 1978; Hayes and Rustioni, 1981; in monkeys: Hutchins et al., 1988; Dum and Strick, 1991; He et al., 1995).

5.2.2 The amygdala
The amygdala has a small number of spinal projecting neurons in the central part of the extended amygdale (EAC), the medial division of the central amygdoid nucleus (CeM), and the anterior basolateral amygdaloid nucleus (BLA) on the ipsilateral side in the retrograde study of the present thesis. This result is similar to studies on the cat and monkey (Mizuno et al., 1985; Sandrew et al., 1986). Labeled neurons in the amygdala were only found on the ipsilateral side after upper and mid cervical injections. To date axonal terminals of these neurons in the spinal cord have not been studied.

5.2.3 The bed nucleus of the stria terminalis
In the present thesis, this nucleus of the mouse has a small number of spinal projecting neurons in the mouse. These neurons are mainly located in the posterolateral part of the medial division of this nucleus (STMPL), forming a line of cells, parallel to axons of the internal capsule (ic). This result is not consistently seen in all mice of the present thesis, this may be due to the fact that the injection site varies from C1 to C3. The other possibility is that the tracer spread to the lower brainstem. It is possible that axonal terminals from these neurons reach only the upper most cervical cord. This finding is consistent with the anterograde study in the cat which found anterogradely labeled axonal terminals only in C1 (Holstege et al., 1985). Inconsistent with the above observation, axonal terminals from this
nucleus terminate in the lumbar cord in the rat (Schwanzel-Fukuda et al., 1984). It would be worthwhile to follow the descending axonal terminals in the mouse by injecting the anterograde tracer to this nucleus.

5.2.4 Hypothalamic nuclei
In the present thesis, the hypothalamus of the mouse has prominent ipsilateral spinal cord projections furnished by the paraventricular nucleus. The majority of labeled neurons in this nucleus are in the posterior part (PaPo) where densely packed labeled neurons form an arch extending towards the ZI. This result is consistent with previous studies in other mammals (in rats: Sawchenko and Swanson, 1982; Hallbeck and Blomqvist, 1999; Pyner and Coote 2000; Condes-Lara et al., 2007; in cats: Miura et al., 1983; Caverson et al., 1984; Holstege 1987; Kausz 1990; in monkeys: Saper et al., 1976; Kneisley et al., 1978). The other group of labeled neurons was found in the lateral hypothalamus of the mouse with spinal projections bilaterally distributed with an ipsilateral predominance. Among the subdivisions, the peduncular part of the lateral hypothalamus (PLH) has the largest number of labeled neurons. The tuberal region (TuLH) and the magnocellular nucleus (MCLH) have fewer labeled neurons.

In the present thesis, medially in the ventromedial hypothalamic nucleus (VMH), a small number of labeled neurons was found adjacent to the lateral part of the retrochiasmatic nucleus which also has a small number of ipsilaterally labeled neurons. This is similar to what has been reported in the rat (Swanson and Kuypers, 1980; Leong et al., 1984a; Schwanzel-Fukuda et al., 1984). Caudally in the posterior hypothalamus of the mouse, a small number of weakly labeled neurons were found in its dorsal part in the present thesis. This nucleus has been found to project to the sacral segment in the rat (Schwanzel-Fukuda et al., 1984) and the number of labeled neurons is much greater than that found herein in the mouse.

Laterally, a few labeled neurons are seen in the ipsilateral subthalamic nucleus in the present thesis. Similar results have only been observed in the monkey after upper cervical injections of a retrograde tracer (Takada et al., 1987; Mizuno et al., 1988), though the majority of labeled neurons in the monkey are on the contralateral side.
5.2.5 Diencephalic nuclei
Spinal projections arise from a number of nuclei in the mouse diencephalon in the present thesis. The majority of these projections arise from ipsilateral nuclei.

The most impressive neuronal groups are the dorsal zona incerta (ZID) and the interstitial nucleus of Cajal (InC). Both have strongly labeled neurons on the ipsilateral side. In addition, the ipsilateral A11 (which might overlap with part of the subparafascicular nucleus) also has a considerable number of labeled neurons which are continuous with labeled neurons in the rostral periaqueductal gray. In the present thesis, they are considered as part of the A11 (based on the tyrosine hydroxylase stain in our lab).

A few labeled neurons were found in the ipsilateral nucleus of the posterior commissure (PCom) in the present thesis. Notably, the magnocellular part of this nucleus has more labeled neurons on the contralateral side.

A small group of labeled neurons were present in the nucleus of Darkschewitsch (Dk) and the nucleus of fields of Forel (F) of the mouse in the present thesis. These results are similar to previous studies in the mouse (Qu et al., 2006), rat (Leong et al., 1984a; Schwanzel-Fukuda et al., 1984), cat (Holstege and Tan, 1988; Isa et al., 1988; Holstege and Cowie, 1989; Satoda et al., 2002; Warren et al., 2008), and the monkey (Castiglioni et al., 1978; Carlton et al., 1985).

A small number of weakly labeled neurons were also noticed in the caudal part of the paraxiphoid nucleus (PaXi) in the present thesis. These neurons are close to the medial part of the zona incerta (ZI). Lateral to the A11, a number of cells are also present in the ipsilateral parafascicular nucleus (PF), especially in the medial and ventral parts in the present thesis. This is different from the anterograde result of the lateral parafascicular nucleus injections. The lateral PF mainly projects to the contralateral spinal cord (Marini et al., 1999). In sagittal sections, a small number of labeled cells are also seen in the area caudal to the PF in the present thesis. This area might be the retroparafascicular nucleus (RPF). So far there are no similar reports in other species.

5.3. Neuronal groups in the mesencephalon
5.3.1 The red nucleus

In the present thesis, this nucleus of the mouse has spinal projecting neurons in its rostral and caudal parts after both cervical and lumbar cord injections of the retrograde tracer and topographic organization is observed. Labeled neurons projecting to the lumbar cord are more ventrolaterally located than those projecting to the cervical cord. This is consistent with previous studies (in mice: Carretta et al., 2001; VanderHorst and Ulfhake, 2006; in rats: Murray and Gurule, 1979; Zemlan et al., 1979; Shiel et al., 1983; Leong et al., 1984a; Schwanzel-Fukuda et al., 1984; Daniel et al., 1987; Shen et al., 1990; Masson et al., 1991; Naso et al., 1993; Wang et al., 1996; de Boer-van Huizen and ten Donkelaar, 1999; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979; Holstege and Tan, 1988; Pong et al., 2002; Satoda et al., 2002; in monkeys: Castiglioni et al., 1978; Kneisley et al., 1978; Carlton et al., 1985).

However, genetic data show that the rostral part of the red nucleus, corresponding to the parvicellular part, belongs to the diencephalon; the caudal part, corresponding to the magnocellular part, belongs to the midbrain (Puelles et al., in press). In situ hybridization against GAD67 and vesicular glutamate transporter 2 gene (vGluT2) (from Allen Brain Atlas website– http://mouse.brain-map.org) defines the boundary of these two parts. The rostral part is positive for both genes, whereas the caudal part is only positive for vGluT2 gene. The location and number of vGluT2 positive neurons are similar to those of the rubrospinal neurons in the present thesis, indicating that vGluT2 positive neurons might be rubrospinal neurons. Another landmark for rubrospinal neurons is the C1QL2 gene expression. There is an obvious overlap between rubrospinal neurons and C1QL2 positive neurons, especially in the caudal part of the red nucleus (in situ hybridization data from Allen Brain Atlas website: http://mouse.brain-map.org). In the rostral part of the red nucleus, some C1QL2 positive neurons may not be rubrospinal neurons. Therefore, the red nucleus is not a homogenous neuron group. It contains discrete groups of neurons, suggesting that the red nucleus play various roles.

A few labeled neurons were seen in the ipsilateral red nucleus and this is verified by the anterograde study of the present thesis. In the anterograde study, ipsilateral axonal terminals were traced down to the thoracic segments and they are similarly distributed in the gray matter as those from the contralateral side, with a lower density. This finding is also reported in the rat (Antal et al., 1992), cat (Holstege 1987), and the marsupial (Warner and Watson,
However, these fibers may originate from the red nucleus contralateral to the injection site because some fibers of passage in the ventral tegmental decussation are also labeled in the present thesis. Another possibility is that some rubrospinal axons cross the midline in the spinal cord and extend to the side ipsilateral to the injection site. In the present thesis, contralaterally labeled axonal terminals are densely packed in lamina 5 and, to a lesser extent in lamina 6 and dorsal lamina 7. Some axonal terminals are also distributed in laminae 8, 9, and 10. A few axonal terminals can cross the midline and extend to the other side. This result is similar to other anterograde studies (in rats: Brown, 1974; Antal et al., 1992; Yasui et al., 2001; Küchler et al., 2002; in cats: Gibson et al., 1984; McCurdy et al., 1987; Holstege 1987; Holstege et al., 1988; Fujito and Aoki, 1995; in monkeys: Shapovalov et al., 1971; Ralston et al., 1988; in marsupials: Warner and Watson, 1972). In the present thesis, in lamina 9, some axonal terminals project directly to the extensor motor neurons in the lateral motor neuron group in both C8-T1 and L5-L6. The monosynaptic projection to cervical and thoracic motor neurons has been widely studied (Holstege 1987; Holstege et al 1988; Küchler et al, 2002), but the projection to lumbar motor neurons is only reported in the monkey (Holstege et al., 1988). This present thesis is the first one to show monosynaptic projections of the red nucleus on the dorsolateral motor neurons in the cervical and lumbar cord and this indicates that the red nucleus is playing an important role in distal limb movement.

5.3.2 The precuneiform nucleus and the mesencephalic reticular formation
The precuneiform nucleus (PrCnF) has spinal projections arising from its medial part in the present thesis. In the retrograde study of the present thesis, labeled neurons in this nucleus are only found after cervical and upper thoracic injections of the retrograde tracer and the number of labeled neurons drops dramatically as injections are placed more caudally. The projection to the spinal cord from this nucleus has not been reported before in any species. However, neurons in the cuneiform nucleus (CnF) have been shown to project to the spinal cord in previous studies (Castiglioni et al. 1978; Satoda et al., 2002). This difference may be explained by the different definition of these nuclei between species. Interestingly, rostral to the PrCnF, some labeled neurons are also observed in the medial part of the mesencephalic reticular formation (mRt) in the present thesis. These neurons seem to be continuous with those in the PrCnF. Labeled neurons in the mRt have been demonstrated in many previous studies (in rats: Leong et al., 1984a; de Boer-van Huizen and ten Donkelaar, 1999; in cats: Cowie and Holstege, 1992; Satoda et al., 2002; Warren et al., 2008; in monkeys: Castiglioni et al., 1978; Nudo and Masterton, 1988; Robinson et al., 1994). In the present anterograde
study, labeled axonal terminals from the PrCnF travel in the ventral and lateral funiculi and terminate mainly in laminae 7 and 8 and, to a lesser extent in laminae 9 and 10. Injections of the anterograde tracer to a more rostral location corresponding to the mRt produce a similar result. Labeled axonal terminals are also distributed in laminae 7, 8, 9, and 10 despite the lower density of these axonal terminals in laminae 7 and 8. This may suggest that the PrCnF and the mRt are similar in spinal cord projections. Since both are located in the putative mesencephalic locomotor region (MLR) (Garcia-Rill et al., 1983; Skinner and Garcia-Rill, 1984; Garcia-Rill et al., 1985; Coles et al., 1989; Allen et al., 1996), they might be the functional nuclei of the MLR. This still needs to be confirmed through functional studies.

5.3.3 Other mesencephalic nuclei
The superior colliculus (SC) in the mouse has a number of contralateral spinal cord projecting neurons in the deep and intermediate gray layers in its caudolateral part in the present thesis. Labeled neurons are aligned parallel to the surface of the SC. This is similar to some previous studies (in rats: Leong et al., 1984a; in cats: Kuypers and Maisky, 1975; Hayes and Rustioni, 1981; Cowie and Holstege, 1992; Satoda et al., 2002; in monkeys: Nudo and Masterton, 1989; May and Porter, 1992). However, in cats (Olivier et al., 1991) and monkeys (Castiglioni et al., 1978), these neurons have been demonstrated to be organized in a columnar pattern. The axonal terminals in the rat and cat spinal cord have been located in the dorsolateral portion of lamina 7, with fewer terminals in lamina 6, 8, and 9 of the contralateral upper cervical cord (in rats: Yasui et al., 1998; in cats: Huerta and Harting, 1982; Cowie and Holstege, 1992). In the anterograde study of the present thesis, when the SC is injected, contralaterally labeled axonal terminals are distributed in the above-mentioned areas in the gray matter of the spinal cord. Furthermore, sparse axonal terminals are also present in lamina 5 in the contralateral spinal cord. The tectospinal pathway seems to be conserved in different species.

In the present thesis, the periaqueductal gray (PAG) of the midbrain in the mouse also has a large number of spinal projecting neurons especially on the ipsilateral side. Densely labeled neurons are mainly located in the lateral part, adjacent to the PrCnF. Fewer labeled neurons were found in the dorsal and medial parts of the PAG. This result is consistent with other studies (in rats, cats, and monkeys: Mantyh and Peschanski, 1982; in rats: Leong et al., 1984a; in cats: Cowie and Holstege, 1992; Satoda et al., 2002). However, the distribution of axonal terminals in the mouse spinal cord in the present study is not consistent with previous studies. In the cat and monkey, descending axons terminate mainly in the lamina 8, medial lamina 7
and, to a lesser extent in lamina 5 (in cats: Mouton and Holstege, 1994; in monkeys: Mantyh 1983). In the anterograde study of the present thesis, though axonal terminals are densely packed in lamina 5 and the dorsal part of lamina 6 or 7, the density of axonal terminals in laminae 8, 9, and 10 is much lower. These axonal terminals are still seen in lower lumbar segments. Comparing the injection site in this thesis to that of the cat, it is clear that the injection site in the present thesis involves the entire mediolateral extent of the PAG, whereas the cat study only involves the lateral part of it and the adjacent mRt or the area corresponding to the PrCnF in the mouse. This indicates the different projecting pattern of the medial and lateral PAG. Since labeled neurons in the PAG are close to those in the PrCnF and the axonal terminal distribution of the lateral PAG (LPAG) is similar to that of the PrCnF as shown in the present thesis. This may suggest that the PrCnF, mRt and LPAG are cooperating in locomotion through their terminals in different segments.

In the present thesis, the Edinger-Westphal (EW) and the medial accessory oculomotor (MA3) nuclei of the mouse have been shown to have a small number of neurons that project ipsilaterally to the spinal cord. This is consistent with previous studies (in rats: Basbaum and Fields, 1979; Leong et al., 1984a; in cats: Loewy and Saper, 1978; Holstege and Tan, 1988; Isa and Sasaki, 1992a; in monkeys: Castiglioni et al., 1978; Carlton et al., 1985; Nudo and Masterton, 1988).

In the present thesis, the dorsal raphe nucleus (DR) of the mouse does not have spinal projections but there are a few neurons dorsal to it, just ventral to the LPAG, they might correspond to the supraoculomotor periaqueductal gray (Su3). These neurons are bilaterally labeled with an ipsilateral predominance. In the only other study in this region of the mouse, the DR and Su3 are not found to project to the spinal cord (VanderHorst and Ulfhake, 2006).

5.4 Neuronal groups in the rhombencephalon

5.4.1 The trigeminal nucleus
In the present thesis, the trigeminal nucleus of the mouse has spinal projections from all its subdivisions except the mesencephalic trigeminal (Me5). The majority of spinal projecting neurons are ipsilateral to the injection site in the principal sensory (Pr5) and the caudal spinal (Sp5C) nuclei. The oral (Sp5O) and the interpolar (Sp5I) spinal trigeminal nuclei have bilaterally labeled neurons. These results are similar to other studies (in rats: Leong et al., 1984a; Phelan and Falls, 1991; Diagne et al., 2006; in cats: Burton et al., 1979; Matsushita et
al., 1981; Matsushita et al., 1982; in rats, cats, and monkeys: Nudo and Masterton, 1988). In
the present study, a small group of labeled neurons is found in the area between the rostral
Pr5 and the motor trigeminal nucleus (5N), this might correspond to the intertrigeminal
nucleus (Int5). Dorsal to the 5N, a group of labeled cells might belong to the supratrigeminal
nucleus (Su5). These nuclei have been studied for their importance in respiration
(Chamberlin and Saper 1998; Radulovacki et al. 2003; Song et al. 2006), but their spinal cord
projections have not been reported.

5.4.2 Vestibular nuclei
All major vestibular nuclei have spinal cord projections in the mouse in the present thesis.
The superior (SuVe) and lateral (LVe) vestibular nuclei mainly project to the ipsilateral cord,
whereas the medial (MVe) and spinal (SpVe) vestibular nuclei project bilaterally to the spinal
cord. These results are similar to what has been found in other studies (in mice: Carretta et al.,
2001; VanderHorst and Ulfhake, 2006; in rats: Watkins et al., 1981; Huisman et al., 1984;
Nudo and Masterton, 1988; Shen et al., 1990; in cats: Kuypers and Maisky, 1975; Hayes and
Rustioni, 1981; in monkeys: Kneisley et al., 1978; Carlton et al., 1985). Though these nuclei
have been well known for their spinal projections, the detailed axonal termination in the
mouse spinal cord has not been investigated.

5.4.3 The solitary nucleus
In the present thesis, this nucleus of the mouse was shown to have bilateral spinal projections
from its ventral, lateral, and commissural subdivisions in the mouse with an ipsilateral
predominance. While this is consistent with some studies (in mice: Auclaire et al., 1999; in
rats: Norgren 1978; Mtui et al., 1993; in monkeys: Carlton et al., 1985), other studies showed
that the majority of the spinal projections arise from the contralateral nucleus (in mice:
VanderHorst 2005; VanderHorst and Ulfhake, 2006; in rats: Leong et al., 1984a; in cats:
Kuypers and Maisky, 1975; Loewy and Burton, 1978). Though anterograde studies have been
done in some species, the labelling pattern still remain controversial. In the rat, an ipsilateral
predominance has been shown (in rats: Norgren 1978; Mtui et al., 1993), whereas a
contralateral predominance has been demonstrated in the cat (Loewy and Burton, 1978). As
noted above, the solitary nucleus can be bilaterally labeled which indicates that this nucleus
either gives off bilateral solitariospinal projections or the solitariospinal tract descends in one
half of the spinal cord and its axonal terminals can cross the midline to the other side.
Therefore, the injection site and the distribution of solitariospinal axonal terminals will
determine the labelling pattern of the neurons.
5.4.4 Locus coeruleus and the subcoeruleus nucleus

In the present thesis, locus coeruleus (LC) of the mouse has bilateral spinal projections with an ipsilateral predominance. Most spinal projecting neurons are located in the ventral part of this nucleus. This is consistent across species (in mice: VanderHorst and Ulfhake, 2006; in rats: Guyenet 1980; Huisman et al., 1984; Leong et al., 1984a; Sluka and Westulund, 1992; in cats: Kuypers and Maisky, 1975; Hancock and Fougerousse, 1976; Basbaum and Fields, 1979; in monkeys: Hancock and Fougerousse, 1976; Kneisley et al., 1978; Carlton et al., 1985). However, the axonal termination in the spinal cord varies in different strains (Clark and Proudfit, 1992; Sluka and Westulund, 1992) and possibly is different between species.

The subcoeruleus nucleus (SubC) of the mouse has bilateral spinal projections with an ipsilateral predominance. But its ventral part, which corresponds to the A5, has a contralateral predominance. In the ipsilateral sagittal sections, labeled cells in this nucleus are aligned in a crescent shape that surrounds the 5N. This is similar to studies in various species (in mice: VanderHorst and Ulfhake, 2006; in rats: Guyenet 1980; Leong et al., 1984a; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979; in monkeys: Kneisley et al., 1978).

5.4.5 Dorsal column nuclei

The cuneate (Cu) and the gracile (Gr) nuclei of the mouse have ipsilateral spinal projections from their rostral part in the present thesis. This is consistent with some previous studies (in rats, cats, and monkeys: Burton and Loewy 1977; Nudo and Masterton, 1988; in rats: Leong et al., 1984a; Shen et al., 1990; Kudo et al., 1993; Vallanueva et al., 1995; in cats: Wada et al., 1993). Different results are also reported. Zemlan et al (1979) showed that most of spinal projecting neurons are on the contralateral side in the rat, while Wada et al (1993) found that spinal projecting neurons are in both the rostral and caudal subdivisions of the cuneate and gracile nuclei in the cat. The level of termination of fibers from these two nuclei is also controversial (in rats, cats, monkeys: Burton and Loewy, 1977; in rats: Villanueva et al., 1995). In the mouse, there have not been comprehensive studies of spinal cord projections from these two nuclei.

5.4.6 The cerebellar nuclei

In the present thesis, the cerebellum of the mouse has contralateral spinal projections from its medial (Med) and interposed (Int) nuclei including the anterior (IntA) and posterior (IntP) subnuclei. This result is similar to other studies (in rats: Leong et al., 1984a; in rats, cats, and monkeys: Nudo and Masterton, 1988; in monkeys: Batton et al., 1977; Asanuma et al., 1980).
Despite of the pivotal role of the cerebellum in locomotion, its descending axonal terminals in the spinal cord have not been well studied.

5.4.7 The parabrachial and Köllike-Fuse nuclei
The parabrachial (PB) and Köllike-Fuse (KF) nuclei are the main components of the dorsal respiratory group (Hayward et al., 2004; Jiang et al., 2004; Song et al., 2006). In the present study of the mouse, they have been shown to have bilateral spinal projections with an ipsilateral predominance. This is consistent with previous studies (in rats: Leong et al., 1984a; in cats: Hayes and Rustioni, 1981; in monkeys: Carlton et al., 1985). Both the medial and lateral parabrachial nuclei have spinal projections in the mouse, similar to findings in the rat and cat (Basbaum and Fields, 1979), though different from those in the monkey (Westlund and Coulter, 1980; Carlton et al., 1985) in which only the dorsal or the medial parabrachial nucleus has spinal projections. This subtle difference might be explained by the anatomical definition of the medial and lateral parabrachial nuclei across species. Descending axonal terminals from the PB and KF terminate not only in the ventral horn but also the dorsal horn (in monkeys: Westlund and Coulter, 1980), suggesting their involvement in pain modulation.

5.4.8 The ambiguus, retroambiguus, and medullary respiratory nuclei
In the present thesis, both ambiguus (Amb) and retroambiguus (RAmb) nuclei of the mouse have contralateral spinal cord projections. Ventral to the Amb, a few labeled neurons are also present in the respiratory nuclei: the rostroventrolateral reticular nucleus (RVL), the Bötzinger complex (Bo), pre-Bötzinger complex (PrBo), and the rostral ventral respiratory group (RVRG). These results are very similar to previous studies (in rats, cats, and monkeys: Nudo and Masterton, 1988; in rats: Leong et al., 1984a; Holstege et al., 1997; Hardy et al., 1998; Ellenberger 1999; in hamster: Gerrits et al., 2004; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979; Lan et al., 1997). The RAmb has been shown to project directly to the motor neurons of the respiratory muscles (Boer et al., 2006), whether the Amb also has such projections is still unknown.

5.4.9 Raphe nuclei
In the present thesis, raphe nuclei of the mouse hindbrain have a considerable number of spinal projections. These neurons form an arch shape with adjacent labeled neurons in the gigantocellular reticular nucleus. Spinal projecting neurons are continuous along the midline in the hindbrain and found in the raphe magnus nucleus (RMg), raphe interpositus nucleus (RIP), raphe obscursus nucleus (ROb), and the raphe pallidus nucleus (RPa). These findings
are similar to what has been reported in previous studies ((in mice: Carretta et al., 2001; Vander Horst and Ulfhake, 2006; Braz and Basbaum, 2008; in rats: Bowker et al., 1981; Leong et al., 1984a; Masson et al., 1991; Allen and Cechetto, 1994; Leanza et al., 1995; in cats: Kuypers and Maisky, 1975; Hayes and Rustioni, 1981; Kausz 1991; Wada et al., 1993; in monkeys: Kneisley et al., 1978; Carlton et al., 1985). The axonal terminals from these raphe nuclei have been known for their monosynaptic projections to the motor neurons in the spinal cord in addition to their projections to the dorsal horn (in rats: Jones and Light, 1990; Gilbey et al., 1995; in cats: Alstermark et al., 1987), suggesting that they do not only play a vital role in pain perception, but also mediate the reaction to pain through its connections with spinal cord motor neurons.

5.4.10 The Barrington’s nucleus
The Barrington’s nucleus (Bar) of the mouse in the present thesis has bilateral spinal projections with an ipsilateral preference. This is similar to published studies in the rat (Russo et al., 2004) and the guinea pig (Kuipers et al., 2007). Axonal terminals from this nucleus not only terminate on parasympathetic neurons in the spinal cord but also in the ventral horn (in guinea pig: Kuipers et al., 2007), suggesting a role in movement.

5.4.11 The paralemniscal nucleus
This nucleus of the mouse has contralateral spinal cord projections in the present thesis with densely labeled neurons seen in the entire nucleus. This is consistent with published studies (in rats: Leichnetz et al., 1978; Watkins et al., 1981; Leong et al., 1984a; Reiner et al., 2008; in cats: Basbaum and Fields, 1979; in monkeys: Carlton et al., 1985). Its axonal terminals have been found coursing in the dorsolateral funiculus (in cats: Basbaum and Fields, 1979; in monkeys: Carlton et al., 1985), possibly terminating in both the dorsal and the ventral horns (in rats: Reiner et al., 2008). This provides the anatomical evidence for its involvement in pain perception (Dobolyi et al., 2002). However, whether it has projections to motor neurons in the spinal cord remains unknown.

5.4.12 Reticular nuclei in the hindbrain
In the present thesis, the hindbrain reticular nuclei of the mouse have a large number of spinal projecting neurons. In the rostral hindbrain, the oral (PnO) and caudal (PnC) pontine reticular nuclei and, to a lesser extent the ventral pontine reticular nucleus (PnV) have a great number of large labeled neurons following spinal cord injections. Consistent with studies in the rat (Leong et al., 1984a) and cat (Kuypers and Maisky, 1975), the contralateral rostral PnO of the
mouse has more labeled neurons than its ipsilateral counterpart in the present thesis. Labeled neurons are more medially located in more caudal sections and they show an ipsilateral preference in the caudal PnO, PnC, and PnV.

Continuous with labeled neurons in the PnC and PnV of the mouse, a greater number of labeled neurons is labeled in the gigantocellular nucleus (Gi) in the present thesis. Its neurons in the alpha (GiA) and ventral (GiV) parts form an arch with labeled neurons in the raphe nuclei. Laterally, these neurons are continuous with labeled neurons in the lateral paragigantocellular nucleus (LPGi). There are more labeled neurons in the ipsilateral nuclei than in the contralateral counterparts. This is consistent with other studies (in mice: VanderHorst and Ulfhake, 2006; in rats: Watkins et al., 1981; Leong et al., 1984a; in cats: Kuypers and Maisky, 1975; Basbaum and Fields, 1979; Hayes and Rustioni, 1981; Kausz 1991; Wada et al., 1993; in monkeys: Carlton et al., 1985). But in the dorsal part of the Gi and the dorsal paragigantocellular nucleus (DPGi), there is a contralateral preference in the labelling in the present thesis, similar to results in rats (Leong et al., 1984a). Axonal terminals of the Gi have been demonstrated to terminate on motor neurons in the rat spinal cord (Holstege 1991; Hermann et al., 2003). In the anterograde experiment in the present thesis, a large number of descending axonal terminals from the PrCnF also terminate on neurons in the Gi, this may suggest that the PrCnF have not only direct projections to the spinal cord but also indirect connections through reticular neurons in the hindbrain. This hypothesis will need to be tested with simultaneous anterograde and retrograde tracer injections to the PrCnF and the spinal cord respectively.

Lateral to the PnC and Gi, the parvicellular reticular nucleus (PCRt) of the mouse has a small number of spinal projecting neurons bilaterally with an ipsilateral predominance in the present thesis. This is similar to findings in other species (in rats, cats, and monkeys-Nudo and Masterton, 1988). The intermediate reticular nucleus (IRt) of the mouse also has a small number of spinal projecting neurons bilaterally with an ipsilateral predominance in the present thesis with increased number of labeled neurons at the level of medullary reticular nuclei. This is similar to findings in the rat (Ellenberger 1999).

In the caudal hindbrain, the dorsal (MdD) and ventral (MdV) medullary reticular nuclei are well labeled in the mouse in the present thesis. These neurons head towards the central canal with labeled neurons in the IRt. There is an ipsilateral preference in labelling. Medially, a
considerable number of labeled neurons was found in the ipsilateral paramedian reticular nucleus (PMn) of the mouse. This is consistent with other studies (in mice: VanderHorst and Ulfhake, 2006; in rats: Leichnetz et al., 1978; Watkins et al., 1981; Leong et al., 1984a; Reed et al., 2008; in cats: Basbaum and Fields, 1979; Hayes and Rustioni, 1981; in monkeys: Kneisley et al., 1978; Carlton et al., 1985).

5.5 Conclusions

In conclusion, this thesis has generated the following novel findings in the mouse:

1. In the retrograde study, the overall distribution of spinal projecting neurons is similar to that of other mammals. The precuneiform and epirubrospinal nuclei were found to project to the mouse spinal cord, which has not been reported previously in any species.

2. In the retrograde study, the precuneiform nucleus was shown to project to the cervical and upper thoracic levels of the mouse spinal cord. In the anterograde study, fibers from the precuneiform nucleus were found to terminate in laminae 7, 8, 9, and 10, with an ipsilateral predominance.

3. For the first time, we showed that spinal projecting neurons in the red nucleus are also located in the diencephalic (parvicellular part) part. Indeed, the majority of rubrospinal neurons belong to the diencephalic part. Examination of the Allen Brain Atlas website (http://mouse.brain-map.org) suggests that rubrospinal neurons are glutamatergic. Examination of the Allen Brain Atlas website also reveals that rubrospinal neurons are marked by C1QL2 and my own data showed that rubrospinal neurons are SMI-32 positive, especially in the midbrain part of the red nucleus. The present thesis specified the termination site of the rubrospinal axons in the mouse: contralateral laminae 5, 6, and the dorsal part of the lamina 7 at all spinal cord levels. A few fibers terminate next to neurons in the dorsolateral part of lamina 9 at levels of C8-T1 (hand motor neurons) and L5-L6 (foot motor neurons).
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