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The role of the nucleus retroambiguus in the neural control of respiration, vocalization and mating behavior

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Chapter 1

General introduction

Many motor actions in our daily life, such as speaking, writing, walking and grasping for a cup of coffee, are voluntary movements, and are controlled by the somatic motor system. However, some of our behaviors, for example crying, screaming and laughing, are the result of emotions. These behaviors are controlled by the emotional motor system. The emotional motor system controls survival behavior of the individual as well as of the species. The periaqueductal gray (PAG) plays a crucial role in this system, since it can be considered as an integrator of basic survival behavior. In order to produce this behavior the PAG uses the nucleus retroambiguus (NRA), among other structures, as a relay to reach the motoneurons. The NRA plays an important role in respiration, vocalization and mating behavior, and sends its fibers to motoneurons that innervate muscles involved in these activities. In order to investigate respiration, vocalization and mating behavior, knowledge about the neural control of these behaviors is required. This chapter describes the central organization of motor control, and presents the aim of this thesis.

Motor system

The motor system consists of three components: the somatic, the emotional and the basic premotor interneuronal system. Both the somatic and emotional motor system control the basic premotor interneuronal system and/or motoneurons themselves (Fig. 1). The motoneurons innervate smooth and striated muscles.

Motoneurons

Motoneurons play an essential role in the motor system. They send their axons to the muscle fibers. All motor activities, for example writing, running and grasping, require contraction of striated and smooth muscles. These muscles are innervated by somatic and autonomic motoneurons respectively.

Somatic motoneurons innervate striated muscles. Motoneurons innervating muscles of head, face and eyes are located in distinct nuclei in the brainstem, such as the facial, hypoglossal, motor trigeminal and oculomotor nuclei. Motoneurons innervating neck muscles are located medially in the ventral horn of the upper cervical cord, and those innervating back muscles in the thoracic and upper lumbar spinal cord. Motoneurons innervating the muscles of arms and legs are present in the lateral and dorsal parts of the ventral horn of the cervical and lumbar enlargements, respectively. In general, the more distal the muscles are located, the more dorsal and lateral the location of their motoneurons in the spinal cord.

So-called autonomic motoneurons innervate the heart muscle, glands, and smooth muscles of the lungs, organs of the alimentary tract, bladder, and blood-vessels, and consist of sympathetic and parasympathetic preganglionic motoneurons. The

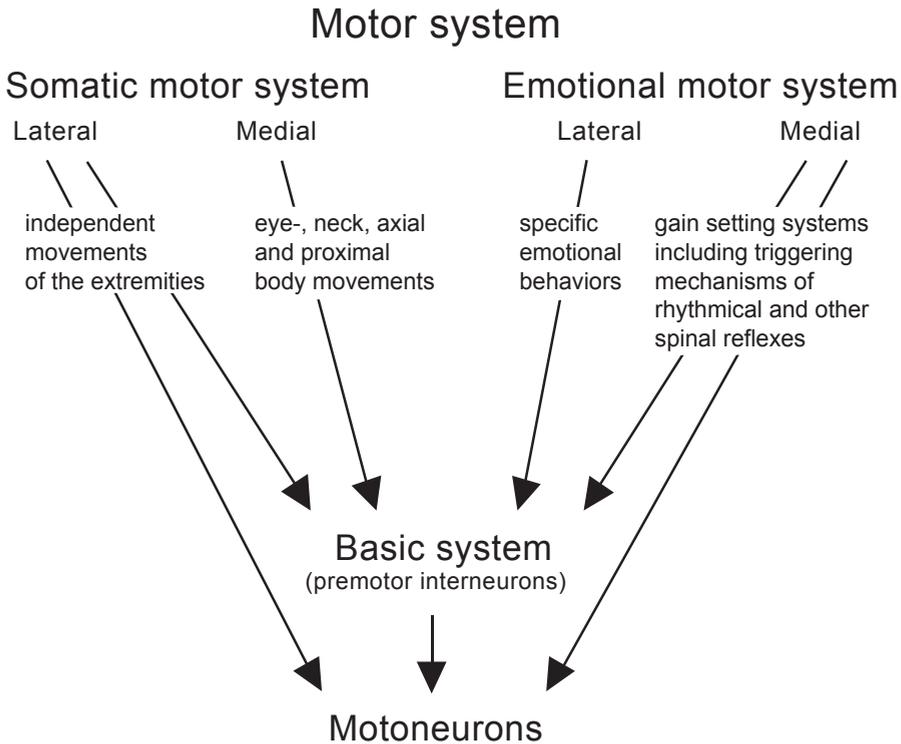


Fig. 1. Overview of the three subdivisions of the motor system (from Holstege, 1996).

sympathetic system increases the heart rate and decreases the activity of the abdominal organs during arousal and physical activity, while the parasympathetic system has the opposite effect during situations of rest and digest. Sympathetic preganglionic motoneurons are located in the intermediolateral cell column in the thoracic and upper lumbar spinal cord. Sympathetic postganglionic neurons are located in the para- and prevertebral ganglia at some distance from their target organs. Parasympathetic preganglionic motoneurons are present in the brainstem in the dorsal motor vagus nucleus, nucleus ambiguus, nucleus Edinger-Westphal, and in the sacral cord in the intermediolateral cell column. The parasympathetic postganglionic neurons are located in peripheral ganglia in or near the target organ.

Basic premotor interneuronal system

The basic premotor interneuronal system consists of premotor interneurons. The somatic and emotional motor system project directly to motoneurons or make use of premotor interneurons to reach the motoneurons. In the spinal cord these premotor interneurons are located in the intermediate zone in laminae V to VIII, and in the brainstem they are located in the lateral reticular formation of the caudal pons and medulla, which can be considered as the rostral extent of the

intermediate zone of the spinal cord. Many premotor interneurons are located in the same segment close to the motoneurons they project to, but some are located at longer distances from their motoneurons. For example, premotor interneurons in the nucleus retroambiguus, located laterally in the caudal medulla oblongata, project to motoneurons in the brainstem and in the lower cervical, thoracolumbar and sacral spinal cord.

Somatic motor system

The somatic motor system controls all voluntary movements of skeletal muscles. This motor system consists of a medial and a lateral component. The lateral component of this system plays an important role in goal directed motor activities of arms and legs, for example grasping a cup of coffee. The main system that produces these goal directed movements is the lateral corticospinal tract. The corticospinal tract originates in neurons in the cerebral motor cortex, whose axons run in the internal capsule, cerebral peduncle and pyramidal tract and finally terminate on interneurons in the lateral reticular formation of the brainstem and in the intermediate zone of the spinal cord. These interneurons, in turn, project to motoneurons that innervate skeletal muscles involved in motor activities. Some of the axons in the corticospinal tract terminate directly on motoneurons in brainstem and spinal cord.

Another system that controls voluntary movements is the rubrospinal tract. This tract originates in the red nucleus, a nucleus which is located in the rostral mesencephalon, and its axons terminate on premotor interneurons and motoneurons of distal fore- and hind limbs. In humans, unlike other mammals, the rubrospinal pathway is not well developed and, therefore, plays only a minor role in the control of voluntary movements.

The medial component of the somatic motor system plays an important role in posture control and maintenance of balance. It controls movements of eyes, head, neck and proximal body. Systems controlling such movements are the vestibulo-, reticulo-, tecto-, interstitio-, and medial corticospinal tract. All these pathways, except the medial corticospinal tract, have their origin in different areas of the brainstem, such as the vestibular nuclei, reticular formation, tectum and the interstitial nucleus of Cajal. The medial corticospinal tract originates in the motor cortex cerebri. All these structures project to medially located motoneurons innervating axial neck- and eye muscles, and to interneurons of the basic premotor interneuronal system.

Emotional motor system

The emotional motor system controls basic survival behaviors such as freezing, aggression, micturition and mating behavior. Like the somatic motor system, the emotional motor system also consists of a medial and a lateral component.

The lateral component is involved in specific emotional behaviors like micturition, vocalization and mating behavior. These activities are mediated by structures in the central nervous system that are part of the limbic system, such as the PAG,

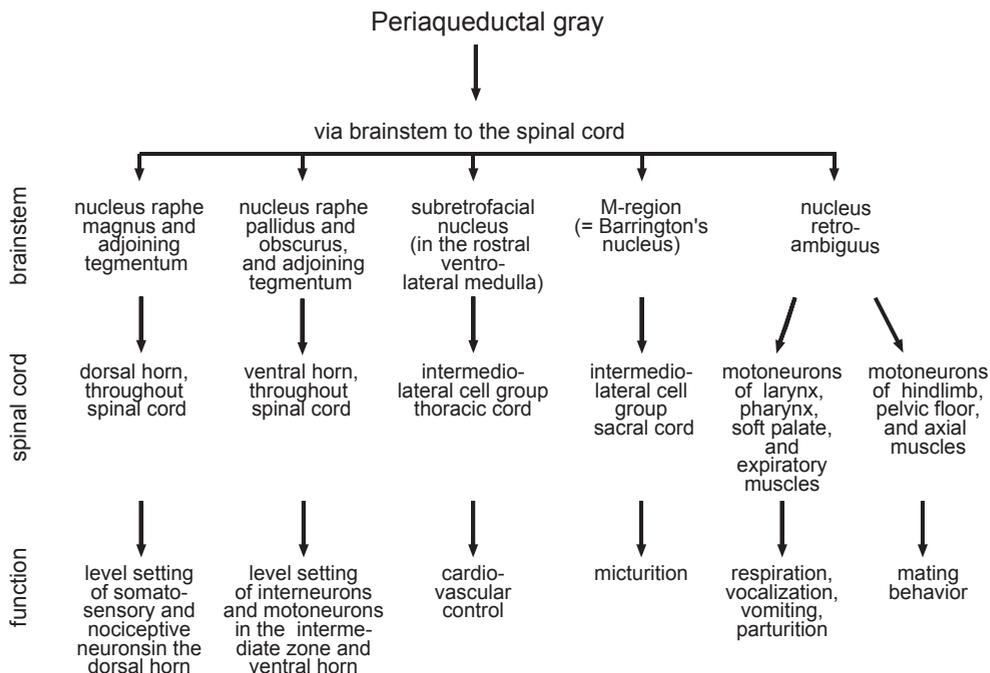


Fig. 2. Overview of the descending projections from the PAG to different regions in the caudal brainstem and spinal cord, and their possible functions.

hypothalamus, amygdala, bed nucleus of the stria terminalis, medial preoptic area and the limbic cortex, which in turn have distinct projections to interneurons of the basic premotor interneuronal system (Fig. 2).

In contrast to the lateral component, the medial component does not have specific projections to interneurons, but projects diffusely to all premotor interneurons and motoneurons in brainstem and spinal cord. The medial component is considered as a level setting system, because it changes in general the excitability of premotor interneurons, sensory- and motoneurons. Cell groups located medially in the pons and medulla play an important role in this system (Fig. 2).

Role of the PAG in the emotional motor system

The PAG can be considered as the most caudal part of the limbic system that organizes complete behaviors in the context of survival of the individual and survival of the species. Electrical stimulation in the PAG in cats resulted in defensive behaviors as aggression or freezing (Bandler and Depaulis, 1991). In humans, stimulation of the PAG elicits feelings of extreme fear (Nashold et al., 1969). Stimulation of the PAG in anaesthetized animals has been shown to produce a strong inhibition of nociception, micturition (Holstege et al., 1986; Blok and Holstege, 1996), vocalization (Holstege, 1989; Zhang, 1994; Davis et al., 1996) and blood pressure changes (Lovick, 1993, 1996). Lesion studies have shown that the PAG is also involved in mating behavior (Sakuma and Pfaff, 1979).

The PAG executes these survival behaviors by way of projections to the ventromedial tegmentum of the caudal pons and medulla for its control of the level setting systems (see Holstege, 1991 for review) including nociception control. The PAG also projects to more specific premotor cell groups as Barrington's nucleus for micturition (Holstege et al., 1986; Blok and Holstege, 1996), the subretrofacial nucleus in the rostral ventrolateral medulla for blood pressure control (Carrive and Bandler, 1991; Lovick, 1993) and the so-called nucleus retroambiguus (NRA) in the caudal medulla for respiration control and vocalization, and possibly also for vomiting and mating behavior (Fig. 2; Holstege, 1989, VanderHorst and Holstege, 1996).

To integrate these motor activities the PAG receives projections from other limbic structures, such as the prefrontal and orbitofrontal cortex, amygdala, bed nucleus of the stria terminalis, hypothalamus (Holstege, 1991), but also from the spinal cord (Mouton and Holstege, 2000).

All these premotor interneuronal cell groups in pons and medulla, which receive projections from the PAG, in turn project to autonomic and somatic motoneurons in brainstem and spinal cord (Fig. 2). Barrington's nucleus, for example, projects to parasympathetic preganglionic motoneurons in the sacral cord innervating the bladder. Via an inhibitory relay in the intermediomedial cell column Barrington's nucleus also inhibits the motoneurons of the external sphincter of the bladder. These motoneurons are located in Onuf's nucleus in the S1-S2 segments. Neurons in the ventrolateral medulla, at the levels between the facial and hypoglossal nuclei, send their axons to sympathetic preganglionic motoneurons in the intermediolateral cell column of the thoracolumbar cord (Ciriello et al., 1986), which neurons innervate the kidneys and adrenal medulla for blood pressure control.

The NRA is located ventrolaterally in the most caudal medulla (Fig. 3). It projects to distinct motoneuronal cell groups in the brainstem and spinal cord, mainly contralaterally. In the brainstem NRA fibers terminate on motoneurons of pharynx and soft palate which are located in the dorsal group of the nucleus ambiguus (Figs. 3 and 4; Holstege, 1989; Boers et al., 2005a), which projection is involved in respiration, vocalization, and vomiting. The NRA projection to the motoneurons of diaphragm in the cervical cord plays an important role in inspiration (Feldman, 1985). For the control of expiration, the NRA sends its fibers to motoneurons of intercostal and abdominal muscles in the thoracic and upper lumbar cord (Figs. 3 and 4; Holstege, 1989). In hamster (Gerrits et al., 1999; 2004), cat (VanderHorst and Holstege, 1995) and rhesus monkey (VanderHorst et al., 2000b), the NRA also projects to a distinct set of motoneurons in the lumbosacral spinal cord innervating axial, hind limb, and pelvic floor muscles (Figs. 3 and 4). These NRA-lumbosacral projections are thought to be involved in mating behavior. In female cats and monkeys, these projections differ greatly in strength depending on whether or not the animal is in estrus (VanderHorst and Holstege, 1997b; VanderHorst et al.,

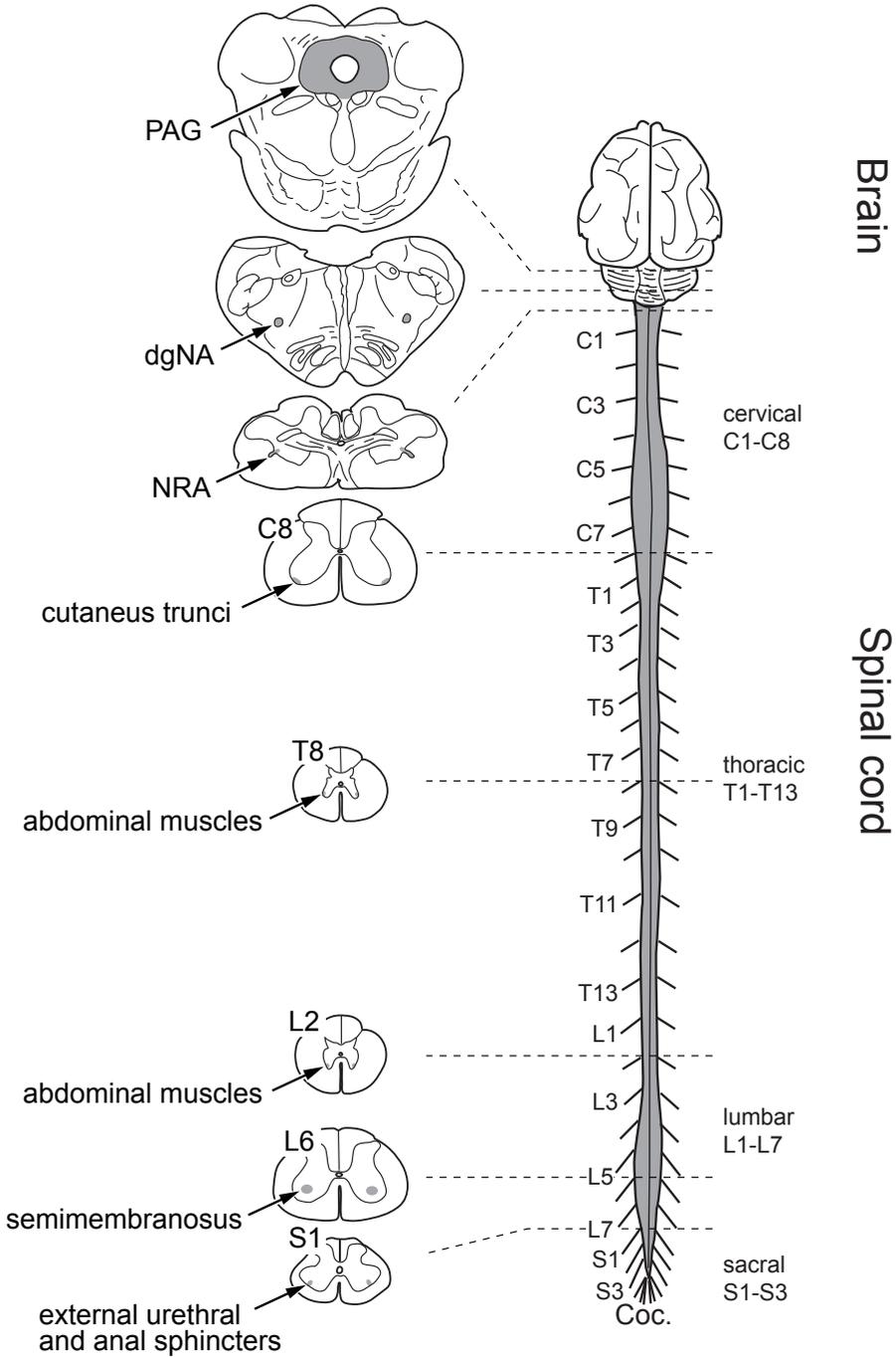


Fig. 3. Representation of the central nervous system of the cat, consisting of the brain and spinal cord. Transverse sections demonstrating the location of the midbrain PAG, the dgNA and NRA in the medulla, and motoneuronal cell groups at five different levels of the spinal cord (dark gray).

2002b). In estrous cats the number of NRA terminals is almost nine times higher than in non-estrous animals, a difference which is caused by axonal sprouting (VanderHorst and Holstege, 1997b).

Aim of the thesis

The work presented in this thesis focuses on the lateral part of the emotional motor system, and in particular on the role of the NRA in abdominal pressure control and mating behavior (Fig. 4). First of all, it presents a complete overview of all NRA-spinal cord projections. It describes the nature of the NRA projections to abdominal and laryngeal motoneurons and shows what structures in the central nervous, other than the NRA, control the motoneurons of pharynx and soft palate. With regard to mating behavior this thesis determines the location of the cells containing the estrogen receptor-alpha and also focuses on the influence of estrogen on the PAG-NRA pathway (Fig. 4). Finally, it attempts to answer the question whether the neurons in the NRA that control mating behavior also control respiration.

The NRA plays an important role in abdominal pressure control in the context of respiration, vocalization and vomiting, and probably also in mating behavior. Although there exists detailed information about specific NRA projections to motoneuronal cell groups in brainstem and spinal cord that are involved in these activities, a complete overview of its efferent spinal projections has never been demonstrated. The anterograde tracing study in chapter 2 presents a complete overview of efferent projections from the NRA to all segments of the spinal cord. Abdominal pressure can be changed by activity of the muscles forming the wall and the bottom of the abdominal cavity, such as the abdominal and pelvic floor muscles. The motoneuronal cell groups of these muscles receive numerous projections from the NRA. The ultrastructural study in chapter 3 shows what the exact nature is of these NRA projections to motoneurons of abdominal external oblique and cutaneus trunci muscles.

Vocalization is the production of sound and is induced by airflow from the thorax along the vocal cords in the glottis opening. This airflow is the result of an increase in abdominal and thoracic pressure caused by contraction of intercostal, abdominal, and pelvic floor muscles. Laryngeal muscles determine the position of the vocal cords. Vocalization can be elicited by stimulation in the PAG. Lightmicroscopical tracing and physiological studies (Holstege, 1989; Zhang et al., 1995) have revealed that the PAG does not project directly to motoneurons of muscles involved in vocalization, but uses the NRA as a relay to excite these motoneurons. The projections from the NRA to motoneurons of laryngeal muscles are difficult to study at the lightmicroscopical level, because these motoneurons are scattered throughout the lateral reticular formation of the medulla. Therefore, the ultrastructural study of chapter 4 determined these direct projections from NRA to laryngeal motoneurons.

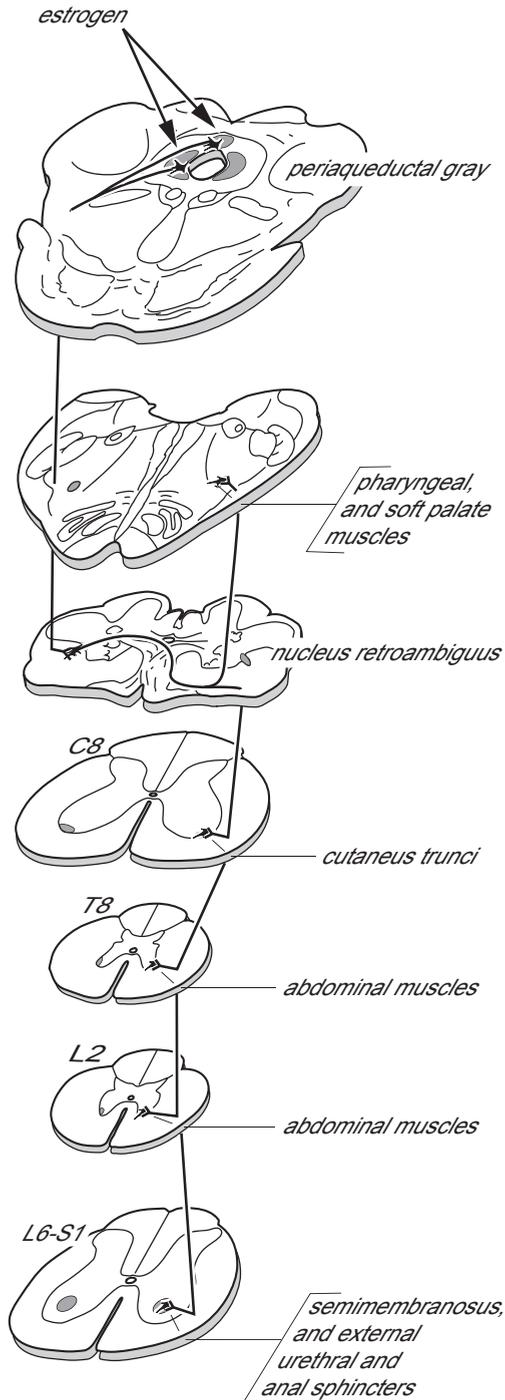


Fig. 4. Overview showing NRA-motoneuronal projections, involved in abdominal pressure control and mating behavior, and the influence of estrogen on the PAG-NRA projection, which are presented in this thesis.

Vocalization and respiration not only involves laryngeal muscles, but also pharynx and soft palate. The motoneurons of both muscle groups are located in the dorsal group of the nucleus ambiguus (dgNA) in the medulla oblongata. These motoneurons can be distinguished at the lightmicroscopical level, because they form a separate group in the lateral tegmental field. It is already known that the NRA has numerous projections to pharyngeal and soft palate motoneurons, but a complete overview of all cell groups in the central nervous system projecting to these motoneurons is still lacking. For that reason a light- and electron microscopic tracing study was done to present all structures in the central nervous system that project to the motoneurons of pharynx and soft palate. This study is described in chapter 5.

Besides abdominal pressure control, the NRA is also involved in mating behavior. It is well known that estrogen plays a crucial role in the display of mating behavior in mammals. The mating posture of the female hamster consists of lordosis of the back and elevation of the tail, and during this posture the hamster is immobile for several minutes. This posture enables the male hamster to mount. The female hamster displays this posture only in estrous periods after stroking the fur of the lower back and perineum. She has a complete estrous cycle of four days, of which the estrous period takes 12-20 hours. When estrogen levels are low and the hamster is not in estrous the female behaves aggressively towards the male hamster, and doesn't allow him to mount.

In female cats mating behavior is more complicated than in hamsters. In cat it consists of lateral deviation of the tail, lordosis of the back and treading of the hind limbs. This behavior is displayed after stroking the lower back, perineum or flanks by a male cat or human individual. Female cats only display mating behavior when they are in estrous, but never in non-estrous periods. They have an estrous cycle that lasts for two to three weeks and they can be in estrous up to eight days. In both hamster and cat it seems that estrogen modulates the function of the neuronal pathway involved in mating, because a similar stimulus, such as stroking the lower back or perineum, does not elicit mating behavior in non-estrous periods in contrast to estrous periods. Since estrogen plays an important role in mating behavior it is interesting to know what structures in the brain are influenced by this hormone. In both hamster and cat the distribution of neurons containing the estrogen receptor-alpha is determined, using immunohistochemical techniques. Chapter 6 describes the distribution of neurons containing the estrogen receptor-alpha in the mesencephalon, pons and medulla oblongata in the female hamster. Chapter 7 presents the distribution of these neurons in the central nervous system, including the complete spinal cord, of the cat. In cat it is already known that estrogen influences the NRA-lumbosacral pathway by inducing sprouting of NRA fibers at the level of the motoneurons in the lumbosacral cord, but the question remains whether estrogen also influences the PAG-NRA-motoneuronal pathway at the level of the PAG itself. In addition to the distribution of neurons containing the estrogen receptor-alpha, chapter 7 also presents a combined retrograde tracing

and immunohistochemical study which determines whether these neurons in the PAG project to the NRA.

As mentioned above, the NRA consists of premotor interneurons that have direct access to motoneurons involved in the control of abdominal pressure and to motoneurons controlling mating behavior. It is not known, however, to what extent the NRA cells assumed to control mating behavior by projecting to the lumbosacral motoneuronal cell groups differ from the NRA cells projecting to abdominal or intercostal motoneurons, or even from those projecting to larynx, pharynx and soft palate. Thus, specific neurons in the NRA might project to mating muscle motoneurons, and other NRA neurons send their fibers to the motoneurons of the abdominal muscles. The study in chapter 8 investigates, in cooperation with dr. P.A. Kirkwood of the Institute of Neurology, London, UK, using electrophysiological techniques whether the neurons in the NRA with projections to the lumbosacral cord, and involved in mating behavior, are the same or differ from those involved in respiration.