Chapter 1

General introduction

General introduction
This thesis presents work that has been performed at the department of Anatomy of the University Medical Center Groningen. Part of the work in this department is focussed on the neuroanatomy of micturition which is the subject of this thesis. This introduction will first describe the motor system and in which manner it controls micturition and subsequently the scope of this thesis will be presented.

Motor system
The motor system, which controls all movements that an individual can make, can be divided into a part that originates from the cerebral cortex which is most important for voluntary control of movements and a part that originates from the limbic system of the brain and is important for involuntary movements. Both the cerebral cortex and the limbic system act on the same set of premotor interneurons and motoneurons that innervate the striated and smooth muscles that produce movements of the body and viscera.

Striated or skeletal muscles, that can move the skeleton, are innervated by somatic motoneurons, while smooth muscles, that can produce movements in the viscera, are innervated by autonomic motoneurons. The autonomic motor system can be further subdivided into a sympathetic and a parasympathetic subdivision. The sympathetic nervous system causes the body to be in an active state while the parasympathetic nervous system gets the body in a resting state. Somatic motoneurons are located in the ventral horn of the spinal cord and in certain brainstem motoneuronal nuclei. Autonomic motoneurons are located in the intermediolateral cell column (IML) of the spinal cord (sympathetic motoneurons: cervical-lumbar spinal cord, parasympathetic motoneurons: sacral spinal cord) and in several brainstem autonomic motornuclei (only parasympathetic motoneurons). In general, somatic motoneurons are mainly innervated by pathways originating from the cerebral cortex while the autonomic motoneurons are mainly innervated by pathways originating from the limbic system.

Premotor interneurons are neurons that project to several motoneurons. These interneurons permit several motoneurons to act in a coordinated fashion. Most interneurons are located in the intermediate zone of the spinal cord but interneurons for the somatic motoneurons in the brainstem are located in the
medullary an pontine reticular formation, which can be seen as the rostral continuation of the intermediate zone of the spinal cord. Most interneurons project to motoneurons which are directly surrounding the interneurons but some exceptions exist. For example, interneurons of the nucleus retroambiguus (NRA) which are important in the control of abdominal pressure are located in the caudal medulla but maintain long descending projections to several groups of motoneurons located at different levels of the spinal cord as far caudal as the sacral spinal cord.

**Motor pathways originating from the cerebral cortex**

Motor pathways that originate from the cerebral cortex can be divided into a medial and a lateral corticospinal pathway and are most important for making voluntary body movements. Both pathways originate from the primary motor cortex which is located in the precentral gyrus of the cerebral cortex. The medial corticospinal tract controls the proximal musculature of the body. Fibers in this tract course in the ventral funiculus of the spinal cord white matter and terminate on interneurons that are located medial in the ventral horn. These interneurons in turn project to the somatic motoneurons of the striated head, back and trunk muscles that are located in the medial part of the ventral horn of the spinal cord.

The lateral corticospinal tract controls movements of the more distal parts of the body i.e. the extremities and is crucial for movements such as walking and grasping objects. Its fibers course in the lateral funiculus of the spinal cord white matter and terminate on interneurons and somatic motoneurons in the lateral part of the ventral horn of the spinal cord. These lateral somatic motoneurons, in turn, innervate the striated muscles of the extremities.

**Motor pathways originating from the limbic system**

In addition to the motor pathways originating from the cerebral cortex, several different motor pathways exist that originate from the limbic system. The movements that are controlled by the limbic system are to a large degree made involuntary and include for example fear expression, smiling, sexual behavior and micturition. Movements of the viscera that are important to maintain body homeostasis are also controlled by pathways that originate from the limbic system. These pathways originate from structures in the limbic system such as the central nucleus of the amygdala, the bed nucleus of the stria terminalis and the lateral hypothalamus and the periaqueductal gray matter (PAG). These limbic structures project, directly and via interneurons, to the somatic and autonomic
motoneurons that innervate the striated and smooth muscles that are necessary to produce movements.

**Micturition**

Micturition is one of the behaviors that is mainly under control of the limbic system, although voluntary cortical control can be exerted over the onset of micturition. Urine is produced continuously in the kidneys, and is then stored in the bladder. When the bladder capacity has been reached, micturition has to take place to empty the bladder. Several parts of the brain, located at different rostrocaudal levels, are involved in the control of micturition. These include parts

![Figure 1. Schematic depiction showing the different nuclei (right side, in gray) that are involved in the central nervous system control of micturition and their rostrocaudal location in the central nervous system (left side).](image-url)
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of the lumbosacral spinal cord, the pons, the midbrain and the hypothalamus (figure 1; de Groat, 1998; Holstege and Mouton, 2003). Micturition is controlled by the central nervous system through a reflex pathway that involves parts of the spinal cord and the brainstem. This micturition reflex pathway is depicted in figure 2 and will now be described in more detail.

Information about the amount of urine in the bladder is monitored by stretch receptors which are located in the bladder wall. This information is conveyed, via Aδ-fibers in the primary afferent division of the pelvic nerve, to the dorsal horn of the lumbosacral spinal cord (Morgan et al., 1981; Janig and Morrison, 1986). Subsequently, this information is relayed to the PAG which is located in the

Figure 2. Schematic depiction of the reflex pathway that controls micturition. The afferent (sensory) limb is depicted on the left side of the figure while the efferent (motorical) limb is depicted on the right side.
midbrain (Blok et al., 1995; Vanderhorst et al., 1996; Keay et al., 1997; Mouton and Holstege, 2000; Klop et al., 2005). The PAG, in turn, projects to a nucleus in the dorsolateral pons, called the pontine micturition center (PMC; Blok and Holstege, 1994). This nucleus is also known as Barrington’s nucleus because it was first described by a British neurologist called Barrington (Barrington, 1925). In rat sensory information from the sacral spinal cord is not only relayed to the PAG but also directly to the PMC (Ding et al., 1997; Blok and Holstege, 2000), but this is not the case in cat (Blok et al., 1995).

The PMC is called the ‘micturition center’ because electrical stimulation in this nucleus can elicit micturition (Holstege et al., 1986). The PMC coordinates the synergistic pattern of muscle activity that is necessary for micturition and as such, can be seen as the set of premotor interneurons that control micturition. In order to micturate, the detrusor muscle fibers in the bladder have to contract and, at the same time, the striated external urethral sphincter muscle has to relax.

The PMC projects to autonomic (parasympathetic) bladder motoneurons, which are located in the sacral IML (Morgan et al., 1979) of the sacral spinal cord. Bladder contractions are elicited by these direct excitatory (glutaminergic) projections on bladder motoneurons in the sacral IML. At the same time, the PMC sends direct excitatory (glutaminergic) projections to interneurons in the intermediomedial cell collumn (IMM). These IMM interneurons, in turn, inhibit the external urethral sphincter motoneurons in Onuf’s nucleus (Holstege et al., 1986; Holstege and Mouton, 2003; Andersson and Wein, 2004) via inhibitory neurotransmitters GABA and glycine (Blok et al., 1997a; Sie et al., 2001). Onuf’s nucleus is located in the ventromedial part of the ventral horn of the sacral spinal cord. It is named after the neurologist Onufrowicz, who has first described it and contains motoneurons for the perineal muscles, including the external urethral sphincter (Onufrowicz, 1899; Sato et al., 1978; Schroder, 1980). The urethral sphincter is the most important muscle that can maintain urethral closure and although it is a striated muscle, there is evidence that the motoneurons in Onuf’s nucleus are not directly innervated by the primary motor cortex (Iwatsubo et al., 1990).

This pattern of projections to different parts of the sacral spinal cord enables the PMC to elicit simultaneous bladder contraction and external urethral sphincter relaxation. When the PMC or the spinal cord is lesioned, as is the case some patients with spinal cord injury for example, coordination between bladder contractions and sphincter relaxation may be lost. These patients exhibit dissynergic micturition, i.e. bladder contractions without sphincter relaxation and often have to be catheterized in order to be able to empty their bladder (Potter, 2005).
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The micturition pathway described above acts as a reflex pathway, i.e. it triggers micturition as soon as the amount of urine in the bladder exceeds a certain threshold level. In healthy subjects this reflex pathway is presumed to be under inhibitory control of higher brain structures. These brain structures can inhibit micturition when it is not desirable, and can initiate micturition when it is desirable and socially appropriate to void. Micturition is thought to be initiated by removal of the inhibition on the reflex pathway. Part of this control of the micturition pathway is thought to be exerted by means of direct projections to the PMC by structures of the limbic system such as the hypothalamus (Holstege, 1987; Valentino et al., 1994; Rizvi et al., 1994; Ding et al., 1999) and bed nucleus of the stria terminalis (Dong and Swanson, 2005). It is likely that these projections are mainly involved in homeostatic control of micturition and that pathways originating from the cerebral cortex are more important for the voluntary control humans can exert over of the onset of micturition. Although the cerebral cortex is known to influence micturition (Gjone and Setekleiv, 1963; Andrew and Nathan, 1964), it is not known whether this control is exerted by means of direct projections to the PMC.

Damage to the higher brain centers that control the micturition reflex pathway may result in symptoms of urge-incontinence, which is also known as overactive bladder syndrome (Andrew and Nathan, 1964; Blaivas, 1982). This form of incontinence is characterized by reduced bladder capacity and increased micturition frequency. Patients who suffer from this syndrome suddenly sense the urge to void, but then often cannot delay micturition until they reach a toilet. The hypothesis is that in these patients the higher brain centers that normally inhibit the micturition reflex pathway are damaged, so that the reflex pathway is functioning on its own, leading to ‘reflexive’ micturition as soon as the bladder is filled. These urge-incontinence symptoms are seen frequently in neurological patients who suffer from lesions or degenerative diseases that also affect brain areas rostral to the PAG, such a Parkinson’s disease (Singer, 1998), Alzheimers disease (Skelly and Flint, 1995) and multiple sclerosis (Chancellor and Blaivas, 1994). Although most of the knowledge about the neuronal control of micturition described above is based on studies that have used different animal models, there is evidence that micturition in humans is controlled by the central nervous system in similar fashion. Symptoms of urinary incontinence in humans have been reported in patients suffering from lesions in brain areas known to be involved in micturition in experimental animals such as the PMC (Sakakibara et al., 1998), the PAG (Sakakibara et al., 1997) or hypothalamus (Yamamoto et al., 2005). In addition, neuroimaging studies in healthy subjects have confirmed the involvement of the PMC, the PAG and the hypothalamus in human micturition (Blok et al., 1997b; Blok et al., 1997c; Nour et al., 2000; Athwal et al., 2001).
Scope of this thesis

In the first part of the thesis (chapters 2, 3 and 4) three studies in cat will be described that present new neuroanatomical data that focuses on brain structures that are involved in the control of micturition by means of direct projections to the PMC (chapters 2 and 3) and on spinal cord afferents that are likely to be involved in micturition (chapter 4). In the last three chapters (chapters 5, 6 and 7), three studies will be presented that describe the areas in the spinal cord and brainstem that control micturition in guinea pigs. The scope of the different chapters will now be described in more detail.

In chapters 2 and 3 of this thesis two cat studies are presented that deal with the question which brain structures control micturition by means of direct projections to the PMC. In chapter 2, a study that systematically describes all afferents to the PMC is presented. The results show that surprisingly few areas of the brain maintain direct projections with the PMC and that micturition thus seems to be under very specific control. Chapter 3 deals with the efferent projections to the brainstem of a part of the anterior cingulate cortex that has often been implicated in the control of micturition. However, the results of this study show that this region does not project to the PMC, but does have a pattern of efferent projections to the brainstem that suggest a role in the fear response. Chapter 4 deals with ascending projections from the sacral spinal cord. This study demonstrates that a part of the sacral spinal cord projects to the PAG, but not to the thalamus. This ascending projection from the sacral spinal cord is likely to relay afferent information from the bladder to the PAG and may be important in bladder sensation.

Chapters 5, 6 and 7 focus on studies in the guinea pig. These studies are important because although most neuro-urological research has been performed in the rat, rat might not be a good animal model for micturition. Urodynamic studies have shown that rat micturition differs significantly from human micturition. During human micturition, the external urethral sphincter relaxes completely during bladder contractions, while in rat the external urethral sphincter contracts rhythmically during bladder contractions (Van Asselt et al., 1995; Walters et al., 2005). The possible function of these rhythmic contractions is to spray urine from the urethra during territory marking behavior (McIntosh et al., 1979). Guinea pigs, on the other hand, void in a manner similar to cats and humans: their external urethral sphincter is completely relaxed during bladder contractions (Van Asselt et al., 1995; Walters et al., 2005), which means that guinea pig micturition may be a better animal model for human micturition. Because nothing is known about the brain areas that control micturition in the guinea pig, the studies presented in chapters 5, 6 and 7 of this thesis investigate the basic neuroanatomical circuitry involved in the control of micturition in guinea pig. These studies can serve as a
basis for further neuropharmacological studies on micturition. First in chapter 5, a retrograde tracing study is presented that describes the location of bladder and urethral sphincter motoneurons. The results indicate that the location of these motoneuronal pools is similar to the location in other species. Thereafter, chapter 6 deals with the location of the PMC or Barrington’s nucleus in the guinea pig. In this study the projections of the PMC to the lumbosacral spinal cord have been studied, as well as the anatomical location of the PMC in relation to nearby nuclei that contain neurons that utilize the neurotransmitter noradrenaline. The results of this study show that the PMC in guinea pig is a nucleus in the dorsolateral part of the pons that is clearly distinct from the noradrenergic nuclei of the dorsolateral pons. The study also shows that the PMC projects to the IML and IMM in the lumbosacral spinal cord in guinea pig. Finally, in chapter 7 a study is described that has investigated neuronal projections from the lumbosacral spinal cord to the PAG in guinea pig. The results show that neurons in the lateral lumbosacral spinal cord project to the central lateral parts of the PAG. This pathway may be important for bladder sensation.