Chapter 8

General discussion

In this thesis several new findings on the neuronal control of micturition have been presented. The first part of this thesis describes three studies in the cat. Chapter 2 describes afferents of the Pontine Micturition Center (PMC). The medial tegmental field, hypothalamus and periaqueductal gray (PAG) were shown to project to the PMC. Moreover, it was demonstrated that the cerebral cortex does not project directly to the PMC. Several functional studies suggest that the medial prefrontal cortex, which includes the infralimbic cortex, is important for the central control of micturition. The third chapter demonstrates, however, that the infralimbic cortex does not project to the PMC but does project to several other parts of the brainstem, including the lateral tegmental field. This projection might be involved in the control of fear behavior. The final study in the first part of this thesis, chapter 4, shows that specific cells in the lumbosacral spinal cord of the cat project to the PAG, but not to the thalamus. This finding has important implications for the understanding of the mechanisms that underlie bladder sensation.

The second part of the thesis describes three studies on the neuroanatomical circuitry that controls micturition in the guinea pig. The importance of these studies lies in the fact that, on urodynamic grounds, the guinea pig may be a much better small animal model for human micturition than the rat, that is currently widely used. The study in chapter 5 of this thesis describes the location of bladder and external urethral sphincter motoneurons in the lumbosacral spinal cord. Furthermore, in chapter 6, it was found that the PMC in the guinea pig does exist and maintains direct projections with specific regions in the lumbosacral spinal cord which allow the nucleus to function as a micturition center. The final study presented in this thesis, in chapter 7, shows that a cell group in the guinea pig lateral lumbosacral cord projects to specific parts of the periaqueductal gray matter. It is likely that these projections relay sensory information from the bladder to the PAG. In this general discussion, the results and implications of the work presented in the previous chapters will be discussed in more detail.

Neuronal control of micturition in the cat

Afferents of the pontine micturition center
Chapter 2 describes a study that is the first to give a complete overview of all PMC afferents. The findings show that only the ventromedial pontomedullary tegmental
field, the ventrolateral and dorsomedial subdivisions of the periaqueductal gray matter (PAG), the perifornical area of the posterior hypothalamus and medial and lateral parts of the medial preoptic area of the hypothalamus project to the PMC. Of these, the diffuse projections from the ventromedial tegmental field of the pons and medulla oblongata have never been described before. These projections are thought to be part of a general ‘level setting’ system, because this part of the tegmental field is known to maintain diffuse projections with the entire central nervous system including the spinal cord.

The projections from PAG to PMC had been described before (Blok and Holstege, 1994). This projection is an important part of the reflex pathway that controls micturition. The study described in this thesis shows that both the ventrolateral and dorsomedial subdivisions of the PAG project to the PMC. Several other studies underline the importance of the ventrolateral PAG in the control of micturition. For example both chemical and electrical stimulation of the ventrolateral PAG can elicit micturition (Matsuura et al., 2000; Taniguchi et al., 2002). Interestingly, it is not known what the influence of the dorsomedial PAG is on micturition.

In addition, the study described in this chapter of this thesis, shows that different parts of the hypothalamus project to the PMC. The PMC receives a specific input from the perifornical area of the posterior hypothalamus. This area is known to contain a dense population of neurons that contain the neuropeptide orexin. The orexinergic neurons in the perifornical area are important for the coordination of feeding, metabolism, and autonomic activity with the control of arousal at the appropriate time of day (Sutcliffe and de Lecea, 2000; Willie et al., 2001). Maybe the pathway from the perifornical hypothalamus to the PMC has a function in coordinating micturition with arousal at the appropriate time of day. Because the orexinergic neurons are wake active (Estabrooke et al., 2001), they may activate the PMC during daytime, stimulating the micturition reflex pathway and thus increase the daytime micturition frequency. At night, this stimulation of the PMC would not take place so that micturition frequency is reduced during sleep.

The study described in this chapter of this thesis, also demonstrated that a medial and a lateral part of the preoptic area of the hypothalamus project specifically to the PMC. This part of the hypothalamus is known to be involved in sexual behavior (Sachs and Meisel, 1988), and therefore the function of the projections from medial preoptic area to PMC may be to inhibit micturition during sexual behavior. This hypothesis is consistent with the fact that it has been suggested that the projection is inhibitory (Rizvi et al., 1998).

Finally, this study shows that several areas that are known to influence micturition such as the amygdala, the bed nucleus (Gjone, 1966) and the medial prefrontal cortex (Gjone and Setekleiv, 1963), do not maintain direct projections with the
PMC. This means that these regions can only influence the PMC by means of projections via structures that do project to the PMC, such as the PAG and the hypothalamus.

**Infralimbic cortical projections to the brainstem**
Chapter 3 describes afferents from the infralimbic cortex to the brainstem. The infralimbic cortex, which corresponds to Brodmann’s area 25, is the ventralmost part of the anterior cingulate cortex.

The infralimbic cortex is an important visceromotor area (Hurley et al., 1991) and several animal studies have suggested the involvement of the anterior cingulate in micturition. For example, stimulation of this area can elicit micturition (Gjone and Setekleiv, 1963) and lesions of the medial prefrontal cortex that include the anterior cingulate impair normal micturition (Matsumoto et al., 2006). Additionally, several neuroimaging studies in healthy human subjects have demonstrated the involvement of the anterior cingulate in micturition (Blok et al., 1997; Sakakibara et al., 1999; Nour et al., 2000; Athwal et al., 2001; Matsuura et al., 2002; Zhang et al., 2005).

Nevertheless, the results from chapter 3 together with those from chapter 2 show that the PMC does not receive any cortical afferents and that the infralimbic cortex does not project, directly, to the PMC. This does not mean that the infralimbic cortex is not involved in the central control of micturition. The infralimbic cortex is known to project densely to both PAG and hypothalamus (Room et al., 1985; Hurley et al., 1991; Freedman et al., 2000; Chiba et al., 2001; Gabbott et al., 2003; Vertes, 2004). These structures, as was shown in chapter 2 of this thesis, project to the PMC. It is likely that the ILc influences micturition via these indirect pathways to the PMC.

The study described in this chapter did demonstrate projections from the infralimbic to the lateral tegmental field of the pons and medulla. These projections have not been described before and may be involved in the control of the expression of fear since the ILc has been shown to be involved in fear behaviour (Quirk et al., 2000; Milad and Quirk, 2002) and the lateral tegmental field contains premotor interneurons that are involved in fear expression.

**Neurons in the lateral sacral cord project to PAG but not to thalamus**
Chapter 4 describes a specific cell group in the lateral part of the sacral spinal cord that projects to the central parts of the lateral column of the PAG, but not to the thalamus.

Based on the fact that primary afferents from the bladder and genitals enter the sacral spinal cord at the location of this cell group, it is hypothesized that these
cells are a relay for sensory information from the bladder and genitalia. The results suggest that this information is relayed directly to the PAG, but not to the thalamus.

The hypothesis that information from the bladder is relayed to the PAG is in agreement with an earlier electrophysiological study that has shown that information from the pelvic nerve, which provides the sensory innervation of the bladder, reaches the PAG (Duong et al., 1999). However, it is not clear what kind of sensory information is conveyed to the PAG via this projection. It might be information about the amount of urine in the bladder. Such information is registered by stretch receptors in the bladder wall and is crucial for the regulation of the micturition reflex. It is also possible that information from the genitals is relayed by this pathway. Finally, it is possible that nociceptive information is relayed to the PAG via this pathway since nociceptive stimuli have been shown to result in C-fos expression in the PAG (Mitsui et al., 2003).

The fact that the lateral cell group does not project to the thalamus, the main structure that relays sensory information to the primary somatosensory cortex, raises the question whether and how information from the bladder enters conscious perception.

Mild distension of the stretch receptors in the bladder registers the amount of intravesical urine. Surprisingly, very little evidence exists about the central processing of non-noxious stimuli from the bladder. In contrast, more studies exist that deal with the central processing of noxious stimuli from the bladder. Physiological and C-fos studies have shown that noxious stimuli ultimately reach the thalamus (Bruggemann et al., 1993; Rodella et al., 1998), but the latencies suggest this is not via direct monosynaptic projections. The lateral cell group in the sacral spinal cord not only projects to the PAG but also the other regions known to be involved in viscerosensory relay such as the parabrachial nuclei (Panneton and Burton, 1985) and the hypothalamus (Katter et al., 1991). Perhaps the visceronociceptive information from the bladder reaches the thalamus via these regions. Somatosensory stimuli, on the other hand, reach the thalamus, and thus the primary somatosensory cortex, via direct thalamic projections of the spinothalamic neurons. This would mean that the central processing of information from the viscera is fundamentally different from somatosensation. This difference is in agreement with a neuroimaging study in humans that shows that non-noxious mild distension of the bladder activates the PAG, but not the thalamus (Athwal et al., 2001).
Neuronal control of micturition in the guinea pig

**Bladder and EUS motoneurons in guinea pig**
Chapter 5 describes the neuroanatomical location of bladder and external urethral sphincter motoneurons in the guinea pig. The study shows that bladder preganglionic motoneurons are located in the intermediolateral cell column of the first and second sacral spinal cord segments. Motoneurons of the external urethral sphincter are located in the medial part of the ventral horn of the first and second sacral spinal cord segments. These locations are in agreement with the locations of these motoneurons in other animals such as rat (Nadelhaft and Booth, 1984; McKenna and Nadelhaft, 1986) and cat (Sato et al., 1978; Morgan et al., 1979; Kuzuhara et al., 1980).

**Pontine micturition center in guinea pig**
Chapter 6 of this thesis describes the location and projections of the pontine micturition center (or Barrington’s nucleus) in the guinea pig. The nucleus is located in the dorsolateral pontine tegmentum, just dorsomedial of the noradrenergic locus subcoeruleus and projects to the lumbosacral intermedialmedial cellgroup (IMM) and to the intermediolateral (IML) cellgroup. The location of the nucleus is similar to that in other animals. Furthermore, the projections of the nucleus to the lumbosacral spinal cord suggest that the nucleus acts as a micturition center in the guinea pig, as it does in other mammals. As such, these projections to the spinal cord form the efferent limb of the micturition reflex pathway.

**Lumbosacral cord projections to PAG in guinea pig**
Chapter 7 describes ascending projections from a cell group in the lateral part of the lumbosacral spinal cord to the central parts of the lateral PAG. Because the lateral part of the dorsal horn of the lumbosacral spinal cord is the region where primary afferents from the bladder enter the spinal cord, these projections may relay information from the bladder to the PAG and are likely to form the afferent limb of the micturition reflex pathway.

**Conclusions about the studies in the guinea pig**
The importance of these studies lies in the fact that the actual micturition behavior in the guinea pig is very similar to that of humans. This is in contrast to micturition behavior in the rat which is the most widely used experimental animal in neurourological research. Urodynamic studies that have compared rat and guinea pig micturition profiles have shown that the EUS muscle of the rat contracts rhythmically while the bladder contracts. However, there is no EUS
activity in the guinea pig during micturition (Van Asselt et al., 1995; Walters et al., 2005). This means that guinea pig micturition is more similar to that of higher species and humans. Therefore guinea pig may be a better small animal model for future neuropharmacological studies aimed at a therapy for the different forms of incontinence. The guinea pig studies described in this thesis describe the basic neuroanatomical circuitry that constitutes the micturition reflex in guinea pig and demonstrate that the found motoneurons and pathways are similar to those known in other species such as rat and cat (de Groat, 1998; Holstege and Mouton, 2003). The guinea pig studies described in this thesis can serve as a basis for future studies on the central control of micturition in the guinea pig.

Epilogue
This thesis presents new neuroanatomical data on the neuronal control of micturition. The neuroanatomical techniques used do not allow to study the precise functions of these neuroanatomical connections. However, the importance of these studies lies in the fact that they can serve as a basis for future behavioral, physiological, molecular biological and pharmacological studies. These studies may contribute to the development of new pharmacological treatments for dysfunctional micturition in general and in particular for overactive bladder syndrome (OAB) which may be caused by loss of inhibition of the micturition reflex pathway.

Until now most of the available treatments for incontinence target the bladder. However, many neurological diseases such as Parkinson’s disease, Alzheimer’s disease, multiple sclerosis and cerebrovascular accidents result in symptoms of overactive bladder syndrome. This indicates that OAB may be, at least in a lot of cases, a neurological in stead of an urological disease. In the future more studies should be aimed at brain areas that control micturition. Pharmacological modulation of these centers may provide an efficient means to treat overactive bladder (OAB).