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

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Chapter 9

General discussion and summary

The emotional motor system controls survival behavior of the individual as well as of the species. The periaqueductal gray (PAG) can be considered as an integrator of basic survival behavior. In order to produce this behavior the PAG does not project directly to motoneurons, but uses structures in the pons and the medulla oblongata as a relay to reach them. One of the relay structures is the nucleus retroambiguus (NRA), located ventrolaterally in the caudal medulla oblongata. The NRA plays an important role in abdominal pressure control, in the context of respiration, vocalization and vomiting, and probably also in mating behavior. The NRA, in turn, sends its fibers to motoneurons, in brainstem and spinal cord, of muscles that are involved in these activities. In the brainstem NRA fibers terminate on motoneurons of pharyngeal and soft palate motoneurons located in the dorsal group of the nucleus ambiguus, which projection is involved in vocalization. For its control in mating behavior, the NRA projects to a distinct set of motoneurons, of axial, hindlimb and pelvic floor muscles, in the lumbosacral cord. These NRA-lumbosacral projections are estrogen dependent, in that they are almost nine times more numerous in estrous than in non-estrous periods. Regarding abdominal pressure control, the NRA sends its fibers to muscles that form the wall and the bottom of the abdominal cavity, such as the abdominal, intercostal and pelvic floor muscles. The NRA is also known as the most caudal part of the ventral respiratory group (VRG) and contains expiratory bulbospinal neurons (EBSNs). EBSNs have excitatory projections to motoneurons of muscles involved in respiration, such as the intercostal and abdominal muscles.

The results of the present thesis provide new information about the role of the NRA in the organization of abdominal pressure control, in the context of respiration and vocalization, and of mating behavior. First of all, this thesis presents a complete overview of efferent projections from the NRA to all spinal cord segments. Relating to abdominal pressure control it describes the nature of its efferent projections to abdominal muscle motoneurons. With regard to vocalization, it describes the nature of NRA efferent projections to laryngeal motoneurons, and it also determines if other structures in the central nervous system control pharyngeal and soft palate motoneurons. Concerning mating behavior, this thesis presents an overview of neurons containing the estrogen receptor-alpha and it investigates the influence of estrogen on the PAG-NRA pathway. Finally, the present thesis distinguishes neurons in the NRA with lumbosacral projections, which are thought to be involved in mating behavior, from neurons that are involved in respiration.

NRA-spinal projections

It has been demonstrated that the NRA projects to a specific set of motoneurons of hindlimb, axial and pelvic floor muscles in the lumbosacral cord, which are thought to be involved in mating behavior. These projections are influenced by estrogen, in that the NRA-lumbosacral projections are more numerous in estrous than in non-estrous periods (VanderHorst and Holstege, 1997b). The idea was that the NRA also plays a important role in the control of the neck, shoulder and forelimb during mating behavior. Therefore, similar NRA projections to specific motoneuronal cell groups in the cervical cord, innervating muscles, which are thought to be involved in mating, were expected. However, the anterograde tracing study in chapter 2 shows that the NRA does not project to a specific combination of neck, shoulder and forelimb muscles motoneuronal cell groups in the cervical spinal cord that could play a role in mating behavior.

In the upper cervical cord the NRA does project to regions containing motoneurons of the cleidomastoid, and of dorsal neck muscles, such as the splenius, biventer cervicis, and complexus. In the lower cervical cord NRA projections were found to phrenic, pectoralis minor and cutaneus trunci motoneuron pools. Probably, these projections do not play a specific role in mating behavior, but they do in respiration or assisting respiration. It might be possible that neurons in the NRA project indirectly to the motoneurons of muscles involved in mating behavior, because projections to interneurons in the intermediate zone were also found. These interneurons, in turn, might project to mating muscle motoneurons in the cervical spinal cord. In the thoracic and upper lumbar cord a bilateral projection was found to abdominal and intercostal motoneurons, and to the dorsomedial ventral horn, possibly containing axial muscle motoneurons and interneurons. The projection to the lumbosacral cord was according to a previous study (VanderHorst and Holstege, 1995).

It is suggested that the posture of the upper body and forelimbs of the female cat during mating behavior is of less importance than that of the lower body and hindlimbs, because direct NRA projections to a specific set of motoneurons of these muscles in the cervical spinal cord have not been found. In contrast, the posture and movements of the pelvis and hindlimbs are thought to be crucial for mating behavior to occur, because there exist monosynaptic excitatory projections from the NRA to a specific set of motoneurons in the lumbosacral cord innervating axial, hindlimb and pelvic floor muscles. It seems that in the cervical and thoracic spinal cord the NRA neurons send their fibers mainly to regions containing interneurons or motoneurons of respiratory and accessory respiratory muscles, but not to motoneurons of muscles involved in mating behavior.

Abdominal pressure control

With regard to abdominal pressure control, in the context of respiration and vocalization, the present thesis provides new information about the exact nature of the efferent projections of the NRA to the motoneurons of the abdominal external oblique, cutaneus trunci and cricothyroid muscles. These NRA-

motoneuronal projections were studied at the ultrastructural level, by combining anterograde tracing of the NRA fibers with retrograde tracing of the motoneurons of the abdominal external oblique (AEO), cutaneus trunci (CTM), and cricothyroid muscles.
Chapter 3 describes the nature of the projections from the NRA to the dendrites of the AEO and CTM. The AEO forms, together with other abdominal muscles, the wall of the abdominal cavity and contractions of this muscle lead to an increase in abdominal pressure, for example during forced expiration, vomiting or coughing. The CTM is a very thin muscle just beneath the skin, whose function is not very clear. From the ultrastructural results it can be concluded that the majority of the NRA terminals make monosynaptic contacts with the dendrites of the CTM and AEO motoneurons. It is suggested that these contacts are of an excitatory nature since the majority of the NRA terminals formed asymmetric synapses with those dendrites and contained mostly round vesicles.

Vocalization
The NRA projections to all motoneuronal cell groups that play a role in vocalization have been studied at the lightmicroscopical level, except those to the laryngeal motoneurons, because these motoneurons are scattered in the lateral tegmental field of the medulla. The electron microscopic study in chapter 4 describes the nature of the NRA projections to the cricothyroid muscle. The results demonstrate that the majority of the NRA terminals make asymmetric synaptic contacts with cricothyroid dendrites and contain pleiomorphic or round vesicles. Similar to the NRA projections to AEO and CTM, these projections are presumably excitatory. The results of chapters 3, 4 and 5, including the results described in previous studies about monosynaptic NRA projections to the semimembranosus (VanderHorst et al., 1997b) and the phrenic motor nucleus (Ellenberger et al., 1990), indicate that, apparently, all of the NRA projections are of an excitatory nature.
Pharyngeal and soft palate motoneurons are located in the dorsal group of the nucleus ambiguus, in the lateral tegmental field of the medulla oblongata. Just as the laryngeal muscles, these muscles also play a role in vocalization and respiration, but also in swallowing and vomiting. The NRA is one of the cell groups controlling these motoneurons. An anterograde and retrograde tracing study in chapter 5 demonstrates that other cell groups with projections to pharynx and soft palate motoneurons are mainly located in the pons, medulla and first cervical segment. In the pons structures projecting to the dgNA are the parabrachial nuclei, and the tegmentum dorsomedial to the superior olivary complex. Within the medulla, neurons with projections to the dgNA are located in the medial tegmental field, the caudal raphe nuclei, the lateral tegmental field, and in the solitary nucleus. It is suggested that the projections from these nuclei in pons and medulla to the dgNA may play a role in vocalization, respiration, swallowing and vomiting or are part of the diffuse level setting system of motoneurons.

Mating behavior

With respect to mating behavior, this thesis describes the localization of estrogen receptor-alpha immunoreactive (ER-α-IR) neurons in the brain and spinal cord, and demonstrates the influence of estrogen on the PAG-NRA pathway by combining estrogen receptor-alpha immunohistochemistry and retrograde tracing.

Estrogen plays an important role in the display of mating behavior. Female cats and hamsters only display mating behavior when they are in estrous. In non-estrous periods they behave aggressively towards the male and do not allow them to mount. In cat is has been shown that estrogen increases the strength of the NRA-lumbosacral motoneuronal projection by almost nine fold (VanderHorst and Holstege, 1997b). This increase is caused by growth of the NRA terminals, since at the ultrastructural level growth-cones were found in the NRA terminals. Since estrogen plays such an important role in mating behavior it is interesting to know which structures in the central nervous system contain the estrogen receptor-alpha and whether other parts of the final common pathway for mating behavior are directly influenced by estrogen due to the presence of the estrogen receptor-alpha.

The distribution of ER-α-IR cells in the brainstem of the hamster and cat are very similar although minor differences exist (chapters 6 and 7). In both species the majority of the ER-α-IR neurons were found in the lateral and ventrolateral parts of the caudal PAG and adjoining tegmentum. Similar to cat, in hamster groups of ER-α-IR cells were also present in the ventrolateral parabrachial nucleus and in the nucleus of the solitary tract. In contrast to the cat, in hamster ER-α-IR cells were also found in the NRA and in the peripeduncular nucleus, but they were not present in the dorsomedial PAG and in the superficial layers of the caudal spinal trigeminal nucleus.

In cat the distribution of ER-α-IR cells has also been studied in the tel- and diencephalon and in the spinal cord. In the tel- and diencephalon ER-α-IR cells have been found in the lateral septal nuclei, amygdala, bed nucleus of the stria terminalis, hypothalamus and medial preoptic area. In the spinal cord ER-α-IR neurons were found in laminae I, II and V, and in the sacral parasympathetic nucleus.

The presence of ER-α-IR cells in the NRA of the hamster is a crucial difference with the cat. Since the NRA is part of the final common pathway for mating behavior, it was expected that in hamster as well as in cat this structure would contain estrogen receptors. Perhaps, in both species different mechanisms are present to activate the descending pathway for mating behavior. In ovariectomized cats, estrogen treatment leads to signs of mating behavior, such as lateral deviation of the tail, lordosis of the back and treading of the hind limbs, after four days. So it seems that it takes four days for cats to come into estrous, which means that it takes four days for the NRA terminals to grow and to change the strength of the NRA-lumbosacral pathway. In hamster the total estrous cycle lasts four days and she is in estous for only 10-20 hours. This period is too short for the development of growth cones and for the NRA terminals to grow. In hamster, the

NRA-lumbosacral pathway is influenced by estrogen at the level of the NRA itself by the presence of estrogen receptors. Possibly, estrogen elevates the excitability of the neurons in the NRA, which project to the mating muscle motoneurons. In cat the NRA-lumbosacral pathway is influenced at the level of the motoneurons in the lumbosacral cord, by inducing growth of the NRA terminals. It is still a question of how estrogen induces the growth of the NRA terminals, because estrogen receptors were not found in any motoneuronal cell group.

The PAG has numerous projections to the NRA, and a great number of estrogen receptors were found in the PAG. Since there the distribution of the cells projecting to the NRA remarkably overlaps with the cells containing estrogen receptors, it was expected that some of the neurons in the PAG, which project to the NRA, contain the estrogen receptor-alpha. However, despite the great overlap between the distribution of both groups of neurons, a relatively small number of NRA-projection neurons in the PAG were ER-α-IR (chapter 7). The majority of them were located dorsally, laterally and ventrolaterally in the caudal PAG. In hamster it has also been demonstrated that neurons in the PAG with projections to the NRA contain the estrogen-receptor-alpha (Gerrits et al., 2000b). 12-26% of the PAG cells projecting to the NRA contained ER-α-IR, and they were mainly located in the caudal half of PAG, forming two separate groups in the lateral and ventrolateral part. Thus in both species the PAG-NRA pathway is directly influenced by estrogen, even though this might be for a minor portion, because the number of NRA-projecting cells containing ER-α-IR are relatively small. These NRA-projecting neurons in the PAG might be involved in postural control during mating behavior. Since a lot of the neurons in the PAG are locally projecting interneurons, it is very likely that some of these interneurons are ER-α-IR and project to NRA-projecting neurons in the PAG. In this way it is possible that estrogen directly influences the PAG-NRA pathway, via NRA projecting neurons that contain the estrogen receptor-alpha, as well as indirectly by ER-α-IR interneurons in the PAG.

It is very unlikely that some of the ER-α-IR PAG cells projecting to the NRA are involved in abdominal pressure control. Activities that involve changes in abdominal pressure, such as forced expiration, vomiting and vocalization, are not dependent on estrogen and take place during estrous and non-estrous periods. Therefore, it is not expected that the NRA-projecting neurons in the PAG, which play a role in these activities, are influenced by estrogen. This corresponds with the data that only a minor part of the PAG cells that project to the NRA are ER-α-IR, and that most of the PAG-NRA neurons are not involved in activities influenced by estrogen. Vocalization can be considered as an independent emotional behavior, but, on the other hand, also as a component of receptive and mating behavior. The differences in vocalization in female cats between estrous (high levels of estrogen) and non-estrous periods (low levels of estrogen), and the estrous cry at the end of mating indicates that these components of vocalization are influenced by estrogen. Therefore, it might be possible that some of the ER-α-IR PAG cells project to NRA neurons that play a role in the control of vocalization.

Functional roles for NRA neurons

The general idea was that within the NRA there exist different groups of neurons with different functional properties. Thus, it was expected that neurons in the NRA with projections to mating muscle motoneurons in the lumbosacral cord differ from those projecting to intercostal and abdominal motoneurons, which are involved in respiration, or even from those projecting to motoneurons of larynx, pharynx and soft palate, which play a role in vocalization. Chapter 8 of this thesis demonstrates by means of physiological techniques the physiological properties of the neurons in the NRA with projections to the lumbosacral cord, and investigates whether these neurons can be distinguished from NRA neurons involved in respiration. The results demonstrate that the NRA projections to the lumbosacral cord, including those to the semimembranosus motor and Onuf's nucleus, involve mainly non-expiratory bulbospinal neurons (non-EBSNs). These neurons can be distinguished from the expiratory bulbospinal neurons (EBSNs), because they are not spontaneously active during normal respiration, show weaker modulation with respiration and are slower conducting than the EBSNs. These data confirm the idea of the existence of different groups of neurons with different functional properties within the NRA. Some of these neurons are involved in respiration and others have different functional roles. It is suggested that these NRA neurons with lumbosacral projections might play a role in mating behavior, and that they receive projections from the ER-α-IR cells in the PAG. Probably, there also exist a different group of NRA neurons that send their fibers to laryngeal, pharyngeal and soft palate motoneurons in the brainstem for its control of vocalization.

A concept is presented for the role of the NRA in the organization of abdominal pressure control, in the context of respiration and vocalization, and of mating behavior. These activities are examples of survival behavior and are controlled by the emotional motor system. They can be considered as separate behaviors, but also as components of different emotional behaviors. For example, changes in vocalization and respiration also occur during arousal, aggression, defensive and mating behavior. The motor component of respiration, vocalization and mating behavior is constituted by specific descending projections from the PAG to the NRA, which, in turn, sends its fibers to motoneurons in brainstem and spinal cord. The autonomic physiology underlying these behaviors is controlled by PAG projections to other relay structures in pons and medulla, for example by its projections to the raphe pallidus and obscures nuclei and the subretrofacial nucleus for the control of the excitability of neurons and blood pressure, respectively. All these behaviors are integrated by neurons in the PAG. Thus, the PAG plays a crucial role in the control of emotional behaviors, because, firstly, it receives many afferent fibers from limbic structures and also from the spinal cord, and, secondly, because it sends its fibers to relay structures in the brainstem. The NRA is just one of these relay structures of the PAG for its control of basic survival behavior.