Discussion

Various studies employing a wide variety of paradigms, including discrete lesions, electrical stimulation, neuropharmacological manipulations, and immediate early gene expression, have been performed to investigate neuronal structures involved in different social behaviours. Nevertheless, the mechanisms underlying the basic recognition processes that enable an animal to recognise conspecifics have not been well characterised. In order to understand the process of social recognition in more cellular and molecular details, we aimed to determine the neural systems primarily involved in the process of individual recognition.

1. AOB activation during social stimulation

The AOB of juvenile stimulated (JS) rats showed considerably increased numbers of cells immunoreactive to the neural activity markers c-Fos, Egr.1, and Arc when compared to control and carvone stimulated (CS) rats. This finding is consistent with previous experiments showing the induction of c-F*os* in the AOB of mice exposed to soiled bedding from intact mice of the opposite sex (Dudley & Moss, 1999; Halem et al., 1999). Furthermore, male Wistar rat urine activates cells in the mitral cell layer of the female AOB (Inamura et al., 1999). Our data concerning the selective neural activation in the AOB of the JS group compared to the control group supports the hypothesis that this area is involved in the process of individual recognition. In contrast to the CS group, which showed an increased number of cells immunopositive to c-Fos in the main olfactory bulb and only few c-Fos immunoreactivity cells in the AOB, the JS rats showed few c-Fos immunoreactivity cells in the MOB and numerous immunoreactive cells in the AOB. These findings strengthen the hypothesis that the individuality signals in the social recognition are of pheromonal nature.

In addition to immunocytochemical data, several other lines of evidence support the hypothesis that the AOB plays a critical role in social recognition. Among those researches, several studies focused on the effects of the neuropeptides oxytocin (OT) and arginine vasopressin (AVP) in social recognition (Dluzen et al., 2000; Dluzen et al., 1998; Ferguson et al., 2001; Ferguson et al., 2000). Oxytocin knock-out mice are unable to recognise

conspecifics (Ferguson et al., 2000). By contrast, injecting OT (Dluzen et al., 2000) or AVP (Dluzen et al., 1998) in the olfactory bulb (OB) of male rats caused prolonged social recognition, i.e., these rats are able to recognise other individuals even two hours after the exposure. In conclusion, our results regarding the neuronal activation in the AOB are in agreement with previous findings concerning IEG expression (Dudley & Moss, 1999; Halem et al., 1999) and neuropharmacological studies (Dluzen et al., 2000; Dluzen et al., 1998; Ferguson et al., 2000). These data suggest that the AOB is involved in the processing of individual recognition in the rat.

1.1 Arc expression in the JS AOB

A marked increase in the number of Arc (activity-regulated cytoskeleton-associated protein)- immunoreactive cells was observed in the AOB of the JS rats. By contrast, only a few Arc-ir cells were observed in the AOB of the control and even carvone-stimulated rats. However, in the AOB of the JS group Arc-immunopositive cells were restricted to the granule cell layer. This expression pattern was different from that of c-Fos immunoreactivity cells, which were observed in the mitral cell layer, granule cell layer and even in the glomerular layer of the AOB. Furthermore, Arc immunoreactivity was observed in both cell bodies and in dendrites. This result is in accordance with the increased number of Arc-immunoreactive cells observed only in the granule cell layer of the accessory olfactory bulb after mating in male rats (Matsuoka et al., 2002) and female mice (Matsuoka et al., 2003).

While the apical dendrites of mitral cells in the glomerular layer of the AOB form synaptic connections with axons from sensory cells in the VNO (Matsuoka et al., 1994), the basal dendrites of the mitral cells form bi-directional dendro-dendritic synapses with granule cells in the granule cell layer (Brennan et al., 1990; Matsuoka et al., 1998). The granule cells also receive centrifugal projections from the posteromedial cortical amygdaloid area (PMCo), an AOB target (Martinez-Marcos & Halpern, 1999). Glutamate released by mitral cells (and probably from PMCo fibres) causes depolarisation of granule cells (Brennan et al., 1990), which, in turn, hyperpolarise mitral cells. Accordingly, the pheromonal memory formation during mating is accomplished by the postsynaptic modification of excitatory synapses between mitral and granule cells (Matsuoka et al., 1997).

In contrast to the classical immediate early gene products, which are actively transferred to the nucleus after synthesis, Arc is observed not only in the nuclei but also in the cell bodies and dendrites. The Arc protein directly influences synaptic functions rather than regulating transcriptions like c-Fos. Arc is rapidly regulated by neuronal activity (Lyford et al., 1995). Its expression reaches its peak at 1.5-2 hours, but remains high until 7-8 hours and reaches its base line after 10-12 hours (Matsuoka et al., 2003). The cell-selective expression pattern of Arc is very important, and its expression pattern suggests that Arc is not merely an activity-dependent IEG.

It is assumed that Arc plays a role in the activity-dependent neuronal plasticity in the hippocampus (Steward et al., 1998) and the cerebral cortex (Lyford et al., 1995). In the latter, Arc also plays a role in the modification of the dendrite structure for interaction with the neuronal cytoskeleton in cortical areas. Arc regulates AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor trafficking (Tzingounis & Nicoll, 2006). Thus, it could be speculated that Arc may play an important role in neuronal plasticity in the AOB. The restriction of Arc-ir cells to the granule cell layer suggests the importance of this circuit and the site of neural plasticity in the mitral/granular synapses.

1.2 AOB granule cells expressing Egr.1

A considerable increase in the expression of Egr.1 was observed in the granule cell layer of the AOB of the socially stimulated group compared to the control rats. Many studies have shown that Egr.1 mRNA is upregulated during different forms of associative learning, and following tetanic stimulation that induces long-term potentiation (Cole et al., 1989; Gong et al., 2005; Grimm & Tischmeyer, 1997; Hall et al., 2000; Nikolaev et al., 1992; Tischmeyer & Grimm, 1999). Behavioural studies have also provided evidence of the expression of Egr.1 following the exposure of rats to novel environments (Hall et al., 2000) or in a learning context (Tischmeyer & Grimm, 1999). Learning-related increases in Egr.1 expression have also been observed in the hippocampus after active avoidance learning (Nikolaev et al., 1992) and brightness discrimination (Grimm & Tischmeyer, 1997). The induction occurs rapidly and is transient. It reaches its peak at 2 hours and its base line after 4 hours (Gong et al., 2005).

Egr.1 activation may constitute a critical mechanism for the encoding of long-lasting memories. In LTP two important steps are critical: the activation of protein kinases and of constitutively expressed transcription factors, and shortly after, the expression of a class of IEGs encoding regulatory transcription factors. As a regulatory transcription factor, Egr.1 interacts with promoter regulatory elements of a host of downstream effector genes. The analysis of Egr.1 DNA binding activity has revealed that the increase in Egr.1 protein following the induction of LTP is associated with increased binding of the protein to its response element (Williams et al., 2000), indicating the functional activation of downstream genes containing Egr.1 response elements. The expression of Egr.1 is necessary for the stabilisation of the later phase of long-term potentiation (Abraham et al., 1993).

1.3 Anterior and posterior parts of the accessory olfactory bulb displayed differential patterns of neuronal activity in the juvenile stimulated group

Exposing male mice to soiled bedding from female mice has resulted in the expression of c-Fos in the anterior AOB, fivefold more than in the posterior AOB (Norlin et al., 2003). In agreement with these results, the juvenile stimulated rats showed more c-Fos-immunoreactive cells in the anterior part of the AOB than in the posterior part. Similar distribution patterns in the anterior AOB were observed for Arc and Egr.1. These findings strengthen the hypothesis that the two subdivisions of the AOB may play different roles in the individual recognition paradigm. The results suggest a predominant activation of receptor cells located in the apical layer of the vomeronasal epithelium. This neuronal activation is associated with pheromones detected mostly by V1R receptors. The prominent expression of c-Fos, Egr.1, and Arc in the anterior part of the AOB suggests an important role of V1R receptor cells and a preferential involvement of the anterior part of the AOB in social recognition. The segregated projections from the anterior and posterior parts of the AOB in the rat (Mohedano-Moriano et al., 2007) and differential patterns of c-Fos induction in these two parts both suggest a role for the anterior AOB in social recognition processing.

2. Amygdala is essentially involved in the pheromonal information processing

Individual recognition is an integrative process, which involves several brain areas, including the amygdala. Increased c-Fos and Egr.1 immunoreactivity was observed in the

amygdala of the JS group when compared to the CS and control groups. Consistent with these results, it has been reported that after creating lesions in the amygdala and hippocampus, the amygdala but not hippocampus is involved in social recognition (Maaswinkel et al., 1996).The amygdala serves an integrative role in behavioural, vegetative and endocrine activities of animals in relation with their environment. It is involved in emotional response (like aggression), integrating input signals (fear conditioning), and initiating related behaviours. Our initial assumption was that vomeronasal amygdala is likely to be involved in social recognition.

Our data concerning increased immunoreactivity in the amygdala indicated the involvement of the amygdala as the first projection target of the AOB in the processing of pheromonal signals during individual recognition. However, the amygdala is not a uniform structure, but a very complex area composed of several nuclei with different cytoarchitecture and a variety of functional subsystems. The individual amygdaloid nuclei and subnuclei appear to play different roles in the modulation of fear, memory, and attention, as well as in some social, reproductive, sexual and sex-related behaviours of rats. Mapping neural activity in different individual nuclei of the amygdala revealed that some nuclei in the vomeronasal amygdala displayed significantly more c-Fos and Egr.1 immunoreactive cells in the JS rats compared to the other rats, which will be discussed below.

2.1 The medial amygdaloid nucleus is involved in the individual recognition processing

A considerable increase in IEGs expression was observed in the VN-amygdala of the JS rats compared to the CS and control groups. Among the individual nuclei in the VN-amygdala, the medial amygdaloid nucleus displayed numerous c-Fos and Egr.1 immunoreactive neurons. These findings are consistent with previous results regarding increased c-Fos immunoreactivity in the medial amygdala of juvenile stimulated mice (Richter et al., 2005). These data strongly support the hypothesis that the medial amygdala may be one of the structures primarily involved in the processing of individual recognition.

The medial amygdaloid nucleus is divided cytoarchitectonically into the anteroventral (MeAV), anterodorsal (MeAD), posteroventral (MePV), and posterodorsal (MePD) part. MeAD and MeAV form the anterior medial amygdaloid nucleus (MeA), and MePD and

MePV form the posterior medial amygdaloid nucleus (MeP). Interestingly, both stimulated groups (JS & CS) displayed considerably increased c-Fos immunoreactivity in MeA (MeAV and MeAD) when compared to the control group. The differences in the pattern of c-Fos expression in the MeA were not significant between the JS and CS rats. By contrast, the number of c-Fos immunoreactivity cells in the MeP of the JS was significantly increased compared to both the control and CS rats.

Consistent with differential neuronal activities displayed by the Me subnuclei, it has been shown that subdivisions of the medial amygdala in male hamsters respond differently to conspecific and heterospecific chemosensory stimulation (Meredith & Westberry, 2004). While the MeA was activated by both stimuli, the MeP was unresponsive to the heterospecific stimulus. Heterospecific and artificial stimulation always activated the MeA and intercalated mass cells (IM), but not the MeP. The immediately adjacent GABA-rich IM, which was activated by all stimuli that suppressed the MeP, apparently suppressed meP (Meredith & Westberry, 2004). But conspecific stimulation always activated both the MeA and MeP. Therefore, it was concluded that the MeA might serve to evaluate different spatiotemporal patterns of input from the AOB and be engaged in excitatory or inhibitory events in the MeP. Accordingly, all these data indicated the involvement of the MeP in social recognition.

The importance of the Me in social recognition was also indicated by studying the effects of oxytocin on social recognition. In the oxytocin knock-out males, bilateral injections of oxytocin into the Me effectively restored species typical recognition responses (Ferguson et al., 2001). Injecting an oxytocin antagonist in the Me of WT males prevented the normal decline in the duration of olfactory investigation. This neuropharmacological study suggested the critical importance of the medial amygdaloid nucleus in the processing of oxytocinergic signals for social recognition. Interestingly, there are no oxytocin binding site in the MeAD, MeAV, and MePV (Veinante & Freund-Mercier, 1997), but there are highly distributed oxytocin binding sites in the MePD. These findings suggest that the MePD be critically involved in integrating social recognition cues and project to related hypothalamic areas involved in behavioural exhibition. In conclusion, while other subnuclei of the medial amygdala may play a role in social recognition, the MePD subnucleus seems to be more intimately involved in social recognition.

In addition to different inputs to the Me subnuclei, differential projections from the Me implies different roles played by each subnucleus. In particular, the axons of the MeAD project through the ansa peduncularis and the stria terminalis to the bed nucleus of the stria terminalis, medial division, posterointermediate part (BSTPI). By contrast, the MePD sends projections over the same pathways that end in the posteromedial subdivision of the BST (BSTPM) and to the medial preoptic nucleus (MPOA). Both circuits (MeAD/BSTPI and MePD/BSTPM) process information important for normal social behaviour, but they regulate different aspects of this behaviour. Olfactory and vomeronasal stimuli reaching the MeAD/ BSTPI circuit produce a general behavioural arousal, enabling the animal to respond to specific signals with appropriate action (Newman, 1999).

2.2 The posteromedial cortical amygdaloid nucleus plays an active role in individual recognition

Significantly increased expressions of c-Fos, Arc, and Egr.1 were observed in the posteromedial cortical amygdaloid nucleus (PMCo) of the JS group. Consistent with these results, it has been shown that in vivo stimulation of freely moving female rats done by species-specific chemosignals released the excitatory neurotransmitter amino acids glutamate and aspartate in the PMCo (Mucignat-Caretta et al., 2006). These data support the hypothesis that the PMCo is an important projection target of the AOB, which is activated by species-specific chemosignals in the individual recognition processes.

The PMCo is one of the VN-amygdala nuclei which receives signals either directly or indirectly from the AOB. The PMCo is involved in social learning (Vochteloo & Koolhaas, 1987) and in the initiation of social behaviours such as sexual behaviour and aggression in rats (Kemble et al., 1984). In primates, the PMCo and the basomedial amygdaloid nucleus (BM) are involved in face recognition and the recognition of other social relevant stimuli (Mumby & Pinel, 1994). Thus, the data presented here strongly support the view that the posteromedial cortical amygdaloid nucleus is an important relay station in neural circuits underlying social recognition.

Furthermore, identifying the glutamatergic/GABAergic nature of immunopositive cells in the PMCo of the JS rats indicated that about 70% of the c-Fos immunoreactivity cells were glutamatergic cells. PMCo receives projections from the AOB through the mitral cell fibres

and projects back to the granule cells in the AOB (in addition to other targets) (Martinez-Marcos & Halpern, 1999). This excitatory feedback to the granule cell layer might be involved in the formation of pheromonal-based social memory during individual recognition.

2.3 Individual nuclei in the bed nucleus of the stria terminalis play differential roles in the process of social recognition

The bed nucleus of the stria terminalis (BST) is a key component of the extended amygdala, a group of anatomically and functionally related structures that extends rostrocaudally from the BST to the central and medial nuclei of the amygdala. The bed nucleus of the stria terminalis is divided roughly into anterior and posterior parts. The cell groups of the anterior part have been arranged into the medial (BSTMA) and lateral (BSTL) groups.

The BST showed an increased neuronal activation during the juvenile stimulation. In contrast to the posterior parts of the bed nucleus of the stria terminalis with only few c-Fos immunoreactivity cells, the medial division, anterior part (BSTMA) displayed significantly increased c-Fos immunoreactivity in the JS compared to the control and CS groups. Conversely, the lateral division of the anterior part of the bed nucleus of the stria terminalis (BSTL) displayed an increased number of c-Fos immunoreactivity cells in the CS group compared to the JS rats. Previous studies have shown that exposure to male soiled bedding (Halem et al., 1999) or to oestrous female urine (Pankevich et al., 2006b) caused significant increase in c-Fos immunoreactivity in the BST. Furthermore, a similar result has been reported for the BST in lactating mice which exhibit a dramatic increase in aggression to a male intruder in comparison to non-aggressive lactating group (Gammie & Nelson, 2001). These data support the hypothesis that the BST is involved in individual recognition.

In contrast to the above-mentioned results (Halem et al., 1999; Pankevich et al., 2006b) reporting c-Fos immunoreactivity in the posterior parts of the BST, the projection site of the AOB and MePD and the location of vasopressinergic cells, the results presented here indicated only a few c-Fos immunoreactivity cells in the posterior BST. Instead, c-Fos immunoreactivity cells were concentrated in the medial division of the anterior part (BSTMA). The role of individual subnuclei of the BST in social recognition is disputable.

Different individual nuclei in the bed nucleus of the stria terminalis are innervated by efferents from various areas. The posterior part of the BST receives projections from the AOB. Amygdalar cell groups associated with various functional systems innervate distinctive regions of the bed nucleus of the stria terminalis. The medial amygdaloid nucleus innervates preferentially the posterior division of the BST. However, the medial nucleus and other amygdalar components of the pheromonal system (posteromedial cortical amygdaloid nucleus) also innervate the medial group of the anterior division. In fact, the BSTMA received projections from AHi, PMCo, MeAD, MePV, and MePD, but the BSTL is innervated by olfactory recipient amygdala, BLP, and Ce (Dong et al., 2001).

In conclusion, while in previous studies (Halem et al., 1999; Pankevich et al., 2006b), different stimuli resulted in an increased c-Fos immunoreactivity in the BSTMPM, in the present study this area did not show a prominent c-Fos labelling after juvenile stimulation. By contrast, the BSTMA displayed increased c-Fos immunoreactivity and thus may be more involved in the juvenile recognition paradigm. This area provides an association between activity in VN-amygdala and hypothalamic neuroendocrine system.

2.4 The olfactory amygdala was activated in both stimulated groups of rats

The olfactory amygdala, which is principally composed of the anterior cortical amygdaloid nucleus (ACO) and posterolateral corticoamygdaloid nucleus (PLCo), receives direct projections from the MOB. The ACo and PLCo are targeted by less intense projections from the AOB (Pro-Sistiaga et al., 2007). In addition, these nuclei receive secondary projections from the medial amygdaloid nucleus, the main vomeronasal recipient area. Through these pathways, the AOB activates the olfactory amygdala. The immunocytochemical findings concerning increased neural activity in the olfactory amygdala in the JS rats were compatible with the anatomical connections.

Juvenile stimulation resulted in neuronal activation and an increased expression of c-Fos and Egr.1 in different individual nuclei of the olfactory amygdala such as ACo and PLCo. These nuclei showed a high expression of c-Fos in both the JS and CS groups of rats compared to the control group. Although it was expected that the olfactory amygdala of the CS rats may show more c-Fos immunoreactivity cells than that of the JS rats, no significant difference in neuronal activity in the olfactory amygdala was observed amongst the two

groups. In concordance with this result, the exposure of male mice to an anesthetised oestrous female in a conditioned place-preference test caused a significant increase in the number of c-Fos-ir cells in the MeA and MePD areas as well as in the ACo and PLCo (Pankevich et al., 2006a). These data suggest that, in contrast to the minor projections from the AOB, secondary projections from vomeronasal amygdala to the olfactory amygdala lead to neural activity in this area.

2.5 The frontotemporal system of the amygdala is not specifically activated in individual recognition

The subnuclei of the basolateral amygdala do not receive main direct projections from the olfactory bulb (Maaswinkel et al., 1996). The insular cortex receiving olfactory input from the primary olfactory cortex and the Me both project to the basolateral amygdala (Coolen & Wood, 1998). While significantly increased c-Fos immunoreactivity was observed in the anterior (BLA) and posterior (BLP) basolateral amygdaloid nuclei in the JS and CS compared to the control rats, there was no significant difference amongst the two stimulated groups. In accordance with these results, lesions restricted to the basolateral amygdala have no effect on the social recognition ability of rats (Maaswinkel et al., 1996). Thus, the basolateral amygdala seems not to be primarily involved in the social recognition process.

2.6 Juvenile stimulated rats displayed increased expression of Arc and Egr.1 immunoreactivity in the amygdala

The analysis of the pattern of Arc expression in the JS group revealed a considerable increase in Arc-immunoreactive cells in the amygdalar subnuclei PMCo and AHi. Although many Arc-ir cells were observed in the MePV and MePD of the JS rats, there was no significant difference noticeable when compared to the CS rats. Only a few Arc-ir cells were observed in the Me of the control group. Consistent with these findings, Arc immunoreactive cells have been observed in the amygdala and hippocampus of rats after electrically induced seizure (Lyford et al., 1995).

The Arc expression in the medial and posterior amygdala, mainly in the PMCo and AHi, demonstrates the involvement of these structures in the information processing during

social recognition. Arc expression is associated with cytoskeletal and synaptic changes in dendrites and synaptic active zones. Therefore, the expression of Arc could enhance the probability and shows the occurrence of synaptic plasticity in these regions of the amygdala in response to social stimulation.

Egr.1 immunoreactivity in the VN-amygdalar system of the JS rats was significantly increased when compared to the CS and control groups. By contrast, there were no prominent differences in the Egr.1-ir expression in the olfactory amygdala of the two stimulated groups of rats. Previous studies have shown different patterns of Egr.1 expression in the individual nuclei of the amygdala according to the stimulus and experimental context (Gong et al., 2005; Grimm & Tischmeyer, 1997; Hall et al., 2000; Nikolaev et al., 1992; Tischmeyer & Grimm, 1999). For example, introducing loser male hamsters to familiar winners caused increased Egr.1 expression in the medial amygdala in the submissive male hamster (Lai, Chen, & Johnston, 2004). The expression patterns of Egr.1-ir in the vomeronasal and olfactory amygdalar functional systems in the JS and CS groups were correlated with stimulus conditions (pheromonal and carvone stimulation, respectively). While JS rats exhibited numerous Egr.1-ir neurons in the VN-amygdala, the patterns of Egr.1 expression in the other parts of the amygdala were similar in the JS and carvone stimulated rats.

2.7 Some similarities were observed in the patterns of c-Fos expression in the amygdala of JS and CS rats

The immunocytochemical analysis in the amygdala of the experimental rats revealed that the VN-amygdala of JS rats displayed remarkably increased c-Fos expression when compared to the VN-amygdala in the control and CS groups. On the other hand, the CS group showed an increased expression pattern of c-Fos in the VN-amygdala compared to the control group. Additionally, while we had expected to find more c-Fos immunoreactivity cells in the olfactory subdivision of the amygdala in the CS compared to the JS rats, the pattern of c-Fos induction was similar in both groups. The reasons for the similarity in the pattern of neuronal activity between the JS and CS rats are:

1- Pheromones/odours of the juvenile stimulus not only contain non-volatile pheromonal molecules which activate the AOB, but also volatile molecules which activate the MOB.

Furthermore, some cells in the vomeronasal organ neuro- epithelium express odorant receptors but project to the accessory olfactory bulb (Levai et al., 2006). Accordingly, the MeA may integrate olfactory and vomeronasal inputs (Licht & Meredith, 1987).

2- The MOB can also respond to some pheromonal signals. Therefore, pheromones not only activate the AOB, but also the MOB (Restrepo et al., 2004). Furthermore, partially increased Fos expression in the VN-amygdala of the CS group can be explained by different internal relations between various amygdalar subnuclei. Tract tracing results showed that the MOB causes only minor projections to the Me. However, there are massive projections to the ACo and the PLCo. The ACo in turn projects to the MeAD and MePV. Also, the ACo and PLCo both project to the AHi and thence to the MePD. Thus, it is not unexpected to see c-Fos-ir in the same vomeronasal amygdalar subnuclei of the CS group.

3.1 The mRNAs encoding for the vesicular glutamate transporters 1 and 2 are expressed in the rat amygdala

Vesicular glutamate transporters (vGLUTs) are responsible for glutamate trafficking and its accumulation in the vesicles. Among the three known vGLUT isoforms, both vGLUT1 and vGLUT2 have been considered definitive markers of glutamatergic neurons, whereas vGLUT3 is expressed even in non-glutamatergic neurons such as cholinergic striatal interneurons.

The results of in situ hybridisation indicate a complementary distribution pattern for vGLUT 1 and vGLUT 2 mRNAs in the amygdala. Expression of vGLUT1 was observed in almost all individual nuclei of the amygdala, except for the Me, Ce, and the BST subnuclei. By contrast, a considerably strong expression of vGLUT2 mRNA was evident throughout the medial amygdaloid nucleus. The PMCo and AHi express both vGLUT1 and 2 mRNAs. A complementary distribution of vesicular glutamate transporters in the rat brain has already been reported (Kaneko & Fujiyama, 2002). Also, the regional expression of vGLUT2 in the medial amygdala has been reported (Hisano et al., 2000). However, so far vGLUT 1 and 2 have not been localised simultaneously in the individual nuclei. The co-expression pattern of c-Fos with vGLUT2 in the MeP as observed here was about 30%, while approximately 70% of c-Fos-ir neurons in the posterior amygdala (PMCo and AHi) were labelled for

vGLUT1 mRNA. Thus, these data support the partially excitatory nature of the activated cells in the medial amygdala during juvenile stimulation. In conclusion, putative glutamatergic projections from the medial amygdala may play a role in the process of individual recognition.

Some authors consider the medial and central amygdaloid nuclei as differentiated parts of the striatum and the rest of the amygdala as cortical structures. According to this categorisation, extrinsic projections from the Me are predominantly GABAergic (like the caudatoputamen), whereas the major projections of the remaining amygdala are not, but are presumably glutamatergic instead (Swanson & Petrovich, 1998). Glutamatergic projections from the MeA innervate the MPOA, another region critically involved in the processing of social recognition (Popik & van Ree, 1991).

3.2 GAD65 and GAD67 mRNAs expression in the rat amygdala

Gamma-amino butyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian brain and is known to be involved in a variety of physiological functions. As the enzyme responsible for the synthesis of GABA, glutamic acid decarboxylase (GAD) plays an important role in nervous system functions. Two forms of GAD, each encoded by a different gene, have been identified (Feldblum et al., 1993). In the cerebellar cortex, GAD65 immunoreactivity has been localised predominantly in axon terminals, whereas GAD67 has been found in the cell bodies and proximal dendrites as well as in many axon terminals of known GABA neurons (Kaufman et al., 1991). Furthermore, GAD67 is more highly concentrated than GAD65 in many GAD-containing cell bodies, whereas GAD65 is more highly concentrated than GAD67 in many axon terminals (Esclapez et al., 1994). However, in many brain regions, the cell bodies that have been immunoreactive for GAD67 were often more numerous than those that were immunoreactive for GAD65. By contrast, the density (quantity) of GAD65 immunoreactive axon terminals was higher than that of GAD67-immunoreactive terminals (Esclapez et al., 1994).

Differential labelling intensities of (strongly to weakly labelled neurons) were observed among different groups of neurons for both GAD65 and 67 mRNA. Our experiment revealed a dense band of labelled neurons that extended ventrally from the striatum and through the central amygdalar nucleus (Ce) to the medial amygdalar nucleus. In general,

the medial and central amygdala exhibited numerous cells labelled for GAD65 and/or GAD 67 mRNA, while in the rest of amygdala the labelling was less dense. A compact group of GAD-expressing neurons was detected in the medial amygdala, especially in the MePD, in the medial column (MePDm). These results are in agreement with previous in situ hybridisation reports (Esclapez et al., 1993). The combination of immunocytochemistry and in situ hybridisation studies demonstrated that a high percentage (about two-thirds) of c-Fos immunoreactivity cells in the MePD and MePV of JS rats are GABAergic. These results imply that the majority of neurons activated during juvenile stimulation are GABAergic. But the analysis of synaptic buttons in the MePD of rats indicated that 91.8% appeared to be excitatory and 8.2% inhibitory (Hermel et al., 2006). To conclude, the majority of GABAergic cells in the Me project to other amygdalar or extra-amygdalar structures. These findings imply that GABAergic neurons which are activated in the Me during social stimulation may give rise to projections to the BST, lateral septum, medial preoptic area, and hypothalamus.

4. Lack of c-Fos immunoreactivity in the vasopressinergic cells in the medial amygdala and BST

To verify the role of the socially active neuropeptides vasopressin and oxytocin in social recognition, the expression of c-Fos in these neurons was studied. Two extrahypotha-lamic sources of vasopressinergic cells are the medial amygdala and BST (de Vries & Miller, 1998). In the medial amygdala, vasopressinergic cells were observed only at the medial border, while c-Fos immunoreactivity cells were observed in the centre of the nucleus. These two populations of neurons did not overlap each other. In the BST, the vasopressinergic cells were localised in the posterior BST, an area devoid of c-Fos immunoreactivity. Fos-ir cells were mainly detected in the anterior part, the medial division of the BST. While vasopressinergic cells may play an important role in social recognition, no immunoreactivity was observed in these cells.

Conclusion

The data presented here indicate an activation of the AOB, especially the anterior part, by social stimulation. Mitral cells, the AOB output cells, project to the amygdala and release glutamate as their neurotransmitter (Fuller & Price, 1988; Hayashi et al., 1993; Trombley &

Westbrook, 1990). In the VN-amygdala, the projection target of the AOB, individual nuclei such as MePD, MePV, PMCo, BSTMA, and AHi were shown to be activated by expressing IEGs c-Fos, Egr.1, and Arc. Many of the c-Fos immunoreactive cells in the MeP were GABAergic and about one-third of them were glutamatergic.

Outlook

While some aspects of the neuronal structures and mechanisms underlying social recognition, social memory and social interaction are known, many details of these mechanisms are still unknown. Although there is some evidence for a deficiency of the oxytocinergic system in autistic patients (Jansen et al., 2006; Modahl et al., 1998) and even some examples of therapeutic effects of oxytocin on autistic patients (Hollander et al., 2007) and social interaction (Kosfeld et al., 2005), animal experiments may not be directly relevant to human social disorders.

Summary

Individual recognition, the animal's ability to recognise and differentiate between familiar and unfamiliar conspecific animals, plays a crucial role in the animal's social behaviour. In rats, individual recognition is mediated by pheromonal signals. To study the neural mechanisms underlying individual recognition, the expression patterns of the IEG product proteins c-Fos, Egr.1, and Arc were investigated immunocytochemically in the brains of socially stimulated rats. For this purpose, adult male rats were previously exposed to juvenile rats in a discrimination test. The results were compared with data from rats which were stimulated by a monomolecular odour, carvone. A third group of rats remained non-stimulated as the control group.

Juvenile stimulated (JS) rats showed a significantly increased expression of c-Fos, Egr.1, and Arc in the AOB in comparison with the control and carvone stimulated (CS) groups. A differential pattern of neural activity was observed in the two parts of the AOB characterised by the expression of different G-protein α subunits $G\alpha i_2$ and Gαo. In the anterior part of the AOB, more cells were activated than in the posterior part of the AOB. The screening of the forebrain for areas specifically activated during social recognition revealed a significantly increased IEG expression in the vomeronasal amygdala of the JS group when compared to the other two groups. A detailed analysis of the neural activity in individual nuclei of the vomeronasal amygdala further revealed a significant increase in c-Fos and Egr.1 expression in the MePD, MePV, PMCo, and AHi nuclei of JS animals. An increased Arc expression was predominantly observed in PMCo and AHi.

Finally, GAD and vGLUT in situ hybridisation was performed to identify the GABAergic or glutamatergic nature of c-Fos-ir neurons. About 30% of the c-Fos-ir neurons in the MePD and 18% in the MePV were GAD65 positive. 38% and 19%, respectively, of the c-Fos-ir neurons in the MePD and MePV were expressing GAD67. Also, 26% of the c-Fos-positive neurons in the MePD and 23% in the MePV were vGLUT2 positive. VGLUT1 expressing neurons were not detected in this area of the amygdala.