

Table of Contents:	Page
I. List of tables.....	4
II. List of figures.....	4
III. List of abbreviations.....	6
Introduction.....	9
1. Advanced mammals live in social groups.....	9
2. Two separate systems detect olfactory signals.....	10
2.1 The main olfactory system preferentially detects highly volatile odorants.....	10
2.2 The accessory olfactory system detects and processes low-volatile odorants.....	11
2.2.1 The receptive area of the accessory olfactory system is the vomeronasal organ.....	11
2.2.2 Main and accessory olfactory bulbs show basically similar cytoarchitectures.....	11
2.3 The two olfactory systems mainly project to different targets.....	12
2.4 The amygdaloid complex, the common target of the two olfactory systems.....	14
3. There are two experimental approaches to investigate social recognition in rodents.....	14
3.1 The Individual recognition paradigm.....	15
3.2 The individual discrimination paradigm	15
4. Neuronal stimulation activates different mechanisms in the cell	16
5. Aims of the project.....	16
Materials and Methods.....	18
1. Materials	18
1.1 Antibodies used in this study	18
1.2 RNA riboprobes.....	19
1.3 Fluorescent conjugates.....	19
2. Method.....	19
2.1 Animal Stimulation	20
2.2 Fixation of animals by vascular perfusion.....	20
2.3 Cresyl violet stain.....	20
2.4 Immunoperoxidase cytochemistry at the light microscopic level.....	20
2.5 In Situ Hybridisation (ISH).....	21

Table of Contents:	Page
2.6 ISH/Immunofluorescence double labelling..	23
2.7 Immunocytochemical analysis	23
Results.....	27
1. Morphological analysis of protein phosphorylation is no promising tool to follow neuronal activation during social interaction.....	27
1.1 Phosphorylated amino acids.....	27
1.2 Visualisation of DARPP-32 and pCREB.....	31
1.3 Investigation of immediate early genes expression	32
2. Exposure of adult male rats to juvenile rats caused expression of c-Fos, Egr.1, and Arc in the accessory olfactory bulb.....	34
3. Juvenile stimulation resulted in different patterns of IEGs induction in the anterior and posterior parts of the AOB.....	37
4. Social stimulation caused high expression of IEGs in the amygdala.....	40
5. The mRNAs encoding for GAD65 and GAD 67, vesicular glutamate transporters 1, and 2, are heterogeneously expressed in the rat Amygdala	52
5.1 Distribution of glutamic acid decarboxylase 65 and 67mRNAs in amygdala.....	53
5.2 Distribution of vGLUT1 and vGLUT 2 mRNAs in amygdala.....	55
6. Co-expression of c-Fos with GADs and vesicular glutamate transporters in the amygdala of the JS group	58
6.1 Co-localisation of c-Fos with GAD65 and 67.....	62
6.2 Co-localisation of c-Fos with vGLUT1 and vGLUT2.....	62
7. Co-expression of c-Fos and oxytocin or vasopressin in the activated regions during social recognition.....	62
Discussion.....	64
1. AOB activation during social stimulation.....	64
1.1 Arc expression in the JS AOB.....	65
1.2 AOB granule cells expressing Egr.1.....	66
1.3 Anterior and posterior parts of the accessory olfactory bulb displayed differential patterns of neuronal activity in the juvenile stimulated group.....	67

Table of Contents:	Page
2. Amygdala is essentially involved in the pheromonal information processing	67
2.1 The medial amygdaloid nucleus is involved in the individual recognition processing.....	68
2.2 The posteromedial cortical amygdaloid nucleus plays an active role in individual recognition.....	70
2.3 Individual nuclei in the bed nucleus of the stria terminalis play differential roles in the process of social recognition.....	71
2.4 The olfactory amygdala were active in the both stimulated groups of rats.....	72
2.5 The frontotemporal system of the amygdala is not specifically involved in individual recognition.....	73
2.6 Juvenile stimulated rats displayed increased expression of Arc and Egr.1 immunoreactivity in the amygdala	73
2.7 Some similarities were observed in the patterns of c-Fos expression in the amygdala of JS and CS rats.....	74
3.1 The mRNAs encoding for the vesicular glutamate transporters 1 and 2 are expressed in the rat amygdala.....	75
3.2 The mRNAs encoding for the glutamate decarboxylase GAD65 and GAD67 were expressed in the rat amygdala.....	76
4. Lack of c-Fos immunoreactivity in the vasopressinergic cells in the medial amygdala and BNST.....	77
Conclusion.....	78
Outlook.....	78
Summary.....	79
References.....	80
Acknowledgements.....	96
Curriculum Vitae... ..	97
Eidesstattliche Erklärung	98

List of tables	Page
2.1 Antibodies used in this study	18
2.2 RNA riboprobes.....	19
2.3 Fluorescent conjugates.....	19
3.1 Summary of statistical analysis for c-Fos expression rats.....	52
3.2 Summary of statistical analysis for Egr.1 expression rats.....	53
List of figures	Page
Figure 1. Cytoarchitecture of the accessory olfactory bulb.....	13
Figure 2. Schematic representation of in situ hybridization with CARD amplification step	24
Figure 3. boxplot.....	25
Fig.4. Patterns of phosphorylated proteins in the AOB of juvenile stimulated rats.....	28
Fig. 5 Patterns of phosphorylated DARPP-32 and CREB proteins in the olfactory bulb.....	31
Fig. 6 Fos expression in the accessory olfactory bulb of three groups of rats	34
Fig. 7 Fos expression in the AOB of three groups of rats.....	35
Fig. 8 Expression of Egr.1 in the AOB of three groups of rats.....	36
Fig. 9 Arc expression in the accessory olfactory bulb of three groups of rats.....	38
Fig. 10 Anterior-posterior distribution patterns of c-Fos, Arc, and Egr.1 immunoreactivity in the AOB of JS rats.....	39
Fig. 11 Quantification of c-Fos immunoreactive cells in the anterior and posterior parts of the AOB.....	40
Fig. 12 Expression of c-Fos in the amygdala in three groups of rats.....	42
Fig. 13 Expression of c-Fos in the BNST of three groups in rats.....	43
Fig. 14 Egr.1 expression in the medial amygdaloid nucleus in three groups of rats	44
Fig. 15 Expression of Arc in the amygdala of three groups of rats.....	46
Fig. 16 Fos expression in the four functional amygdalar subsystems in three groups of rats	47
Fig. 17 Egr.1 expression in the four different amygdalar subsystems in three groups of rats.....	48
Fig.18 Fos expression in individual nuclei of the VN-amygdala of three groups of rats	49

List of figures	Page
Fig. 19 Egr.1 expression in the individual nuclei of the VN-amygdala in three groups of rats.....	50
Fig. 20 Comparative distribution patterns of GAD65, GAD67, vGLUT2, and vGLUT1 mRNA at the level of medial amygdala	54
Fig. 21 Co-expression of c-Fos and GAD65 mRNA in the posterodorsal medial amygdaloid nucleus (MePD) in the JS group of rats	56
Fig. 22 Co-expression of c-Fos and GAD65 mRNA in the posteroventral medial amygdaloid nucleus (MePV) in the JS group of rats	57
Fig. 23 Co-expression of c-Fos and GAD67 mRNA in the posterodorsal medial amygdaloid nucleus (MePD) in the JS group of rats	59
Fig. 24 Co-expression of c-Fos and vGLUT2 mRNA in the posterodorsal medial amygdaloid nucleus (MePD) in the JS group of rats.....	60
Fig. 25 Percentage of Fos protein co-expression with mRNAs encoding GAD65, GAD67, vGLUT1, and vGLUT2 in the posterodorsal and posteroventral parts of the medial amygdala of juvenile stimulated rats.....	61

Abbreviations:**Neuroanatomic structures:****AA** anterior amygdaloid nucleus,**ACo** anterior cortical amygdaloid area,**AH** anterior hypothalamus**AHi** amygdalohippocampal transitional
area**AOB** accessory olfactory bulb**AOS** accessory olfactory system**BAOT** bed nucleus of the accessory
olfactory tract**BL** basolateral nucleus of the
amygdala**BLA** anterior basolateral nucleus of the
amygdala**BLP** posterior basolateral nucleus of
the amygdala**BM** basomedial nucleus of the
amygdala**BMA** anterior basomedial nucleus of
the amygdala**BMP** posterior basomedial nucleus of
the amygdala**BST** bed nucleus of the stria terminalis**BSTIA** bed nucleus of the stria
terminalis, intraamygdaloid division**BSTL** bed nucleus of the stria
terminalis, lateral division**BSTLD** bed nucleus of the stria
terminalis, lateral division, dorsal part**BSTLI** bed nucleus of the stria
terminalis, lateral division,

intermediate part

BSTLJ bed nucleus of the stria
terminalis, lateral division,
juxtacapsular part**BSTLP** bed nucleus of the stria
terminalis, lateral division, posterior
part**BSTLV** bed nucleus of the stria
terminalis, lateral division, ventral
part**BSTM** bed nucleus of the stria
terminalis, medial division**BSTMA** bed nucleus of the stria
terminalis, medial division, anterior
part**BSTMP** bed nucleus of the stria
terminalis, medial division, posterior
part**BSTMPI** bed nucleus of the stria
terminalis, medial division,
posterointermediate part**BSTMPL** bed nucleus of the stria
terminalis, medial division,
posterolateral part**BSTMPLM** bed nucleus of the stria
terminalis, medial division,
posteromedial part**BSTMV** bed nucleus of the stria
terminalis, medial division, ventral
part

BSTS bed nucleus of stria terminalis, supracapsular part	MeAV anteroventral subdivision of Me
Ce central amygdaloid nucleus,	MeP posterior subdivision of Me
CPu caudate putamen	MePD posterodorsal subdivision of Me
DG dentate gyrus	MePV posteroventral subdivision of Me
EL ependymal layer	MHb medial habenular nucleus
Endo endopiriform nucleus	MOB main olfactory bulb
EPL external plexiform layer	MOS main olfactory system
GL glomerular layer	MPN medial preoptic nucleus
GP globus pallidus	MPO median preoptic nucleus
IG internal granular layer	MPOA medial preoptic area
IM intercalated mass,	MS medial septum
IPL internal plexiform layer	OB olfactory bulb
La lateral nucleus of the amygdala	OTF olfactory tract fibres
LaDL dorsolateral portion of lateral nucleus of the amygdala	PA Posterior Amygdala,
LaVM ventromedial part of the lateral nucleus of the amygdala	PAA piriform-amygdalar Area
LaVL ventrolateral part of the lateral nucleus of the amygdala,	PAG periaqueductal gray
LH lateral hypothalamus	PIR piriform cortex
LHb lateral habenular nucleus	PLCo posterolateral cortical amygdaloid nucleus,
LOT lateral olfactory tract	PMCo posteromedial cortical amygdaloid nucleus
M mitral cell layer	Tu olfactory tubercle
Me medial amygdaloid nucleus,	VN vomeronasal nerve
MeA anterior subdivision of Me	VNL vomeronasal nerve layer
MeAD anterodorsal subdivision of Me	VNO vomeronasal organ
	VNS vomeronasal system

Antibodies and biochemical materials:**Biochemical materials:**

aP-ShaDig: alkaline phosphatase sheep anti Digoxigenin

AF 488-GaR: Alexa Fluor 488- goat anti rabbit

AF 594-GaR: Alexa Fluor 594- goat anti rabbit

AF488-SA: Alexa Fluor 488- anti streptavidin

AF594-SA: Alexa Fluor 594- anti streptavidin

Arc: activity- regulated cytoskeletal- associated protein

ARG-VP: Arginine vasopressin

B-MaDig: Biotinylated mouse anti Digoxin

BCIP/NBT: Bromo-chloro-indolyl-phosphate/ nitro blue tetrazolium

B-GaR: Biotinylated goat anti rabbit

B-HaM: Biotinylated horse anti mouse

BSA: Bovine serum albumine

c-Fos: cellular Finkel-Biskis-Reilly murine osteosarcoma

DAB: Diaminobenzidine

DARPP-32: dopamine and cAMP- regulated phosphoprotein- 32 kDa

Dig: Digoxin

Egr.1: Early growth response gene 1

GAD: glutamic acid decarboxylase

Narp: neuronal activity regulated pentraxin

PBS-A: bovine serum albumin in PBS

pCREB: phosphorylated cAMP response element binding protein

P-SA: peroxidase streptavidine

pSer: phosphorylated serine

pThr: phosphorylated threonine

pTyr: phosphorylated tyrosine

vGLUT: veicular glutamate transporters