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der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

DISSERTATION

The Mammalian Lateral Habenular Complex – Projection and Back Projection to the Ventral Tegmental Area, the Center of the Reward System

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

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aus Berlin

Datum der Promotion:

02. März 2018

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1. Summary

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1.1. Abstract

Mammalian behavior largely depends on the individual and its reaction to a stimulus. In principle it is based on two systems, either deeply enrooted reflex-type mechanisms or freely selected actions, which often in the broadest sense regulate "homeostatic" necessities. Such behaviors per se are emotionally neutral. To make an individual perform such psychologically "indifferent" actions nature has developed a specialized brain circuit, the reward system. It predominantly consists of the dopaminergic and serotoninergic midbrain nuclei and their major projection targets, the nucleus accumbens and the medial orbitofrontal cortex. Not the biological necessity, but the grandiose internal feeling after such homeostatic activities like feeding or sexual intercourse makes individuals to perform such acts over and over. For the purpose of counterregulation, there must be an opposing system, which spoils hedonic feelings in unsatisfactory situations. This "anti-reward-system" in the vertebrate brain is represented by the habenula nucleus and its medial (MHb) and especially in its lateral (LHb) complexes. This processing center for multitudinous inputs evaluates a given situation and appropriately decreases dopaminergic activity in the ventral tegmental area (VTA) which leads to an omission of hedonic feelings. To complete the anti-reward signaling cycle, there must be some type of feedback from the VTA to the LHb. In the present investigation the connections from the LHb to the VTA, the back projection from the VTA to the LHb, and the direct and indirect projections from the LHb to the VTA were analyzed in the rat.

Anterograde and especially retrograde tracing in combination with immunocytochemistry and in-situ hybridization experiments of markers for individual transmitter systems disclosed that most neurons in the LHb were glutamatergic and their axons mainly terminated on GABAergic neurons of the ventral midbrain. Anterior and posterior parts of the VTA and the rostromedial tegmental nucleus (RMTg) receive topographically distinct afferents from the LHb. The back projection from the VTA to the LHb is not predominantly GABAergic, as thought previously, but dopaminergic neurons in the VTA substantially contribute to this pathway.

The habenula often is considered as relay station for a reciprocal link between limbic and midbrain areas, while it is not clear, whether these connections are unequivocally reciprocal.

Our data indicate that this actually is the case. Furthermore, we have shown that there are two pathways from the LHb to the VTA: a direct projection from the LHb to the VTA and an indirect one from the LHb via the RMTg to the VTA, which may subserve different biological purposes.

1.2. Abstrakt

Das Verhalten von Säugetieren ist abhängig von dem Individuum selbst und seinen Reaktionen auf äußere oder innere Stimuli. Grundsätzlich basiert es auf zwei Systemen, die im weitesten Sinn homöostatische Erfordernisse regulieren: tief verwurzelte Reflexe oder frei(willig) vollführte Handlungen. Homöostatisches Verhalten ist per se emotional neutral. Damit das Individuum solche indifferenten Handlungen vollzieht, hat die Natur spezielle Neuronenkreise im Gehirn entwickelt, die unter dem Begriff "Belohnungssystem" zusammengefasst werden. Es besteht aus monoaminergen Kernen im Mesencephalon und ihren Zielregionen. Nicht die biologische Notwendigkeit, sondern der großartige Gefühlszustand bei oder nach solchen homöostatisch notwendigen Verhaltensweisen wie Nahrungsaufnahme oder Geschlechtsverkehr treibt Individuen dazu, diese Handlungen zu vollziehen. Natürlich muss ein Gegenspieler existieren, der lustvolle Gefühle in nicht zufriedenstellenden Situationen verhindert. Dieses "Anti-Belohnungssystem" wird durch den Habenularkomplex mit seinen medialen und insbesondere seinen lateralen (LHb) Anteilen repräsentiert. Dieses komplexe Verarbeitungszentrum bewertet die Situation und reduziert gegebenenfalls die Aktivität der dopaminergen Neurone in der Area tegmentalis ventralis (VTA). Um die Anti-Belohnungs-Signalschleife zu schließen muss es eine Rückkopplung von der VTA zum LHb geben.

In der vorliegenden Arbeit wurden die Verbindungen vom LHb zur VTA, die Rückprojektion von VTA zum LHb, sowie die direkten und indirekten Projektionen vom LHb zur VTA am Beispiel der Ratte untersucht. Mit Hilfe von anterogradem und vor allem retrogradem Tracing in Verbindung mit Immunzytochemie und in-situ Hybridisierung mit Markern für spezielle Transmittersysteme konnten wir zeigen, dass die meisten Neurone im LHb glutamaterg sind und ihre Axone überwiegend an GABAergen Neuronen des ventralen Mesencephalons enden. Topographisch differenzierte Efferenzen vom LHb ziehen zur anterioren und posterioren VTA und dem rostromedialem tegmentalen Nucleus (RMTg). Die Rückprojektion von VTA zum LHb ist nicht überwiegend GABAerg, sondern dopaminerge Neurone der VTA tragen maßgeblich zu diesem Signalweg bei. Die Habenula wird oft als Schaltstelle für reziproke Verbindungen zwischen limbischen und mesencephalen Arealen betrachtet, wobei nicht klar ist, ob diese Verbindungen im eigentlichen Sinne reziprok sind. Unsere Daten bestätigen jedoch, dass dies der Fall ist. Des Weiteren konnten wir zeigen, dass es zwei Signalwege vom LHb zum dopaminergen Mesencephalon gibt: eine direkte Projektion zur VTA und eine indirekte, die über den RMTg zur VTA verläuft. An welchen unterschiedlichen biologischen Funktionen die beiden Wege beteiligt sind, ist noch nicht ausreichend geklärt.

1.3. Introduction

Behavior is defined as the sum of events originating from a living organism that is perceptible to its environment. Behavior therefore largely depends on the circumstances of the individual and its reaction to a stimulus and is also potentially controlled by the "Free Will" of the subject [4]. Mammalian behavior in principle is based on two distinctive systems [5]. The first one depends on deeply enrooted reflex-type mechanisms, from simple postural reflexes [6] to complex systems, such as the regulation of blood pressure and oxygen tension [7]. Such important mechanisms have an autonomous regulation and are largely independent from cognitive influences. The second system influences or even controls behaviors, which for humans generally are attributed to the "Free Will" of the subject [4]. Surprisingly, many of these behaviors represent in the broadest sense regulated "homeostatic" activities. These necessities include drinking, feeding, sexual intercourse, or caring for the offspring, all inherent activities, which from a biological point, ensure the survival of the subject and its species. Such behaviors are also hardwired but adaptable to specific environmental cues. In opposite to reflexes, these behaviors are performed voluntarily, when the situation is intrinsically evaluated as appropriate [4]. This second system, however bears the problem that fulfillment of behavioral needs comes without obvious incentives. Why should any individual, animal or human being, perform actions, which per se are emotionally neutral (like feeding or even sexual intercourse), or possess potential gains that may not be achieved in the near future? For this important purpose evolution has developed a specialized brain circuit, the reward system [8, 9]. Its origins predominantly consist of the dopaminergic (DA) and serotoninergic midbrain nuclei and its major target brain nuclei are the nucleus accumbens and the medial orbitofrontal cortex [10]. This system which already exists in zebrafish [11], has evolved with the sole purpose of connecting hedonic feelings to in the broadest sense "homeostatic" but otherwise unincentivized actions. Not the biological necessity, but the grandiose internal feeling after task completion makes individuals to subserve such needs then and in the future. A hallmark of complex and highly regulated systems is that for every force that drives such systems in one direction, there must be a counterforce that pushes back. Research results from the last two decades only, have indeed revealed, there is an opposing system to the reward-enforcing power, which omits hedonic feelings (negative reward) in unsatisfactory situations. A congruent set of data [12, 13, 14] indicates that this "anti-reward-system" in the vertebrate brain is mainly localized in the habenula nucleus and its medial (MHb) and especially in its lateral (LHb) complexes. Subsequent to the recognition of an unfavorable situation the habenula is activated [15, 16, 17] and provokes negative reward via its inhibitory

effects on dopaminergic cells in the VTA and serotoninergic neurons in the rostral raphe nuclei. In contrast to our basic understanding of this principle, knowledge on the detailed network organization is scarce. The LHb requires information, whether a homeostatic action is connected with an unpleasant outcome. To gather such complex information, various direct inputs from the prefrontal cortex [18, 10] and the hypothalamus [19, 20] are necessary. The LHb as processing center for these multitudinous inputs evaluates the situation and appropriately decreases dopaminergic activity in the VTA, resulting in reward omission. To complete the anti-reward signaling cycle, there must be some type of feedback from the VTA to the LHb. Consequently, we have analyzed (i) the connections and potential effects from the LHb to the VTA [2], including (ii) the differential projections to the anterior VTA, the posterior VTA, and the indirect pathway from the LHb via the RMTg to the VTA [3] and (iii) the back projection from the VTA to the LHb [1].

1.4. Material and Methods

1.4.1. Chemicals and Animals

Chemicals were obtained from Sigma, Taufkirchen, Germany, if not indicated otherwise.
Sources of antibodies are given in the corresponding reports.
Adult male and female Wistar rats, weighing 230-320 g (n=14 [1]; n=17 [2]; n=46 [3]) were obtained from an institutional breeder (Department for Experimental Medicine (FEM), Charité University Medicine Berlin). Animals were housed in group-cages under controlled temperature (22 °C) and illumination (12 hour cycle) with water and food ad libitum. After surgery, animals were kept warm until fully recovered from anesthesia and then kept individually under the above conditions.
All animal experiments were approved by the Regional Berlin Animals Ethics Committee and conducted in strict accordance with the European Communities Council directive 86/609/EEC regarding care and use of animals for experimental procedures.

1.4.2. Tracer injections

Tracer injections were performed as described recently [21]. In short, rats were deeply anesthetized by intraperitoneal injection of a mixture of 45% ketamine hydrocholoride (100 mg/ml; CuraMED, Germany), 35% xylazine hydrochloride (20 mg/ml, BayerVital, Germany) and placed into a stereotaxic frame (David Kopf Instruments, Tujinga, CA). For retrograde tracing, Fluorogold (FG; 1% 2-hydroxy-4.4-diamino-stilbene in 0.1 M cacodylate buffer, pH 7.4; Fluorochrome, Denver, CO), subunit B of cholera toxoid (CtB; 1% in distilled water; List Biological, Campbell, CA) or WGA- apoHRP- gold (10-20 µg/ml in distilled H2O, pH 7.0 - 7.5; E-Y Laboratories, Inc., San Mateio, CA, USA) were unilaterally injected into selected areas of the LHb, VTA or RMTg. Tetramethylrhodamin conjugated dextran amine (TMR-DA; 2.5% in distilled water, pH 7.4; Molecular Probes, Eugene, OR) was used for anterograde controls. Stereotaxic coordinates were adapted according to the atlas of Paxinos and Watson (1998) [22].

1.4.3. Fixation of animals by vascular perfusion

After 3 to 14 days of survival rats were again deeply anaesthetized by intraperitoneal injections of a cocktail consisting of 45 % ketamine (100 mg⁄ml; Ketavet), 35% xylazine (20 mg⁄ml; Rompun) and 20 % saline, at a dose of 0.16 ml⁄100 g of body weight, supplemented by 200 IU heparin intraperitoneal. Subsequently, animals were fixed via transcardial perfusion with PGPic (4 % paraformaldehyde, 0.05 % glutaraldehyde, and 0.2 % picric acid in 0.1 M phosphate buffer,

pH 7.4; [23]). Brains were dissected out, cryoprotected in 0.4 M sucrose for about 4 h and in 0.8 M sucrose overnight, cut into blocks at preselected rostro-caudal levels, shock-frozen in hexane at -70 °C, and stored at -80 °C until use.

1.4.4. Immunoperoxidase cytochemistry at the light microscopic level

Freely floating cryostat sections (25 µm) were treated for 15 min with 1% sodium borohydride in phosphate buffered saline (PBS) and thoroughly washed in PBS. Thereafter, sections were pretreated for 30 min in a blocking and permeabilizing solution, consisting of 10% normal goat serum (NGS; Interchem, Bad Kreuznach, Germany), 0.3% Triton X-100 (Serva, Heidelberg, Germany), and 0.05% phenylhydrazine (Merck, Darmstadt, Germany) in PBS at room temperature (RT). Primary antibodies were applied in PBS containing 10% NGS, 0.3% Triton X-100, 0.1% sodium azide, and 0.01% thimerosal for 36 h at 2 °C. Sections were washed, treated for another 24 h with the secondary antibody and subsequently exposed for 12 h to an avidin-biotin-elite complex solution (1:200 in PBS, Vector Laboratories, USA). After preincubation for 15 min in a solution of 0.05% diaminobenzidine (DAB) and 10 mM imidazole in 50 mM Tris buffer, pH 7.6, the visualization of the bound peroxidase was started by the addition of 0.0015% hydrogen peroxide and stopped after 15 min at RT by repeated washings with PBS. Sections were mounted onto gelatin-coated slides, air-dried not longer than 30 min, dehydrated through a graded series of ethanol, transferred into xylene, and coverslipped with Entellan (Merck, Darmstadt, Germany).

1.4.5. Immunofluorescence cytochemistry

Freely floating cryostat sections (25 µm) were treated as above. Subsequent to the primary antibody sections were exposed for 24 h to fluorochrome-labeled secondary antibodies (Alexa488-labeled goat anti-rabbit immunoglobulin G (IgG) and/or Alexa594-labeled goat anti-chicken IgG; Invitrogen, Karlsruhe, Germany), diluted 1:2000 in 0.3% Triton X-100 and 0.1% sodium azide at RT. After repeated washings in PBS sections were mounted onto gelatin-coated slides, shortly air-dried, coverslipped with MountFluor (Biocyc GmbH, Luckenwalde, Germany), and analyzed with a Leica CS-SL confocal microscope.

1.4.6. Immunoperoxidase cytochemistry at the electron microscopic level

For electron microscopy cryostat sections were treated freely floating as above with the following exceptions. Only the blocking and permeabilizing solution contained Triton X-100 (0.1%), whereas the subsequent solutions were devoid of detergent. Immunoreacted sections

were postfixed in 2% Osmium tetroxide (OsO4), dehydrated in series of increasing ethanol concentrations, and embedded in araldite [24]. Semithin sections and interposed ultrathin sections were cut with a Leica Ultracut microtome. Semithin sections were stained with 1% toluidine blue, pH 9.3, and photographed with a Leica DMRB photomicroscope. Ultrathin sections were stained with uranyl acetate followed by lead citrate [25] and examined with a Zeiss EM912 electron microscope.

1.4.7. Preembedding-immunogold cytochemistry at the electron microscopic level

For immunogold cytochemistry, sections were treated as above with the following exceptions. After treatment with the primary antibodies sections were thoroughly rinsed, preincubated in a buffer consisting of 0.1% carboxylated bovine serum albumin (BSA-C, Biotrend, Köln, Germany), 0.1% Tween-20, and 0.1% sodium azide in PBS for 1 h at room temperature. Gold-labeled secondary antibodies, diluted 1:50 in the same buffer, were applied overnight in a cold room. Next morning, sections were rinsed twice in PBS (containing 0.1% Tween-20) and then twice in PBS. Gold-labeled secondary antibodies were postfixed in 2% glutaraldehyde in PBS, and again washed several times in PBS. After silver intensification and gold-toning (see below) sections were postfixed in 2% OsO4, dehydrated in series of increasing ethanol concentrations, and flat embedded in araldite [24].

1.4.8. Preembedding double label immunocytochemistry at the electron microscopic level

For this purpose, sections were simultaneously treated with two primary antibodies of different species as in the immunoperoxidase procedure above. Next steps followed the immunogold protocol, again using biotinylated and gold-labeled secondary antibodies simultaneously. After postfixation with glutaraldehyde, the corresponding rinses bound peroxidase activity was visualized as above, followed by silver intensification and gold-toning (see below).

1.4.9. Silver intensification of colloidal gold and stabilization of the silver coat by gold-toning

Prior to immunocytochemical processing sections were rinsed in PBS and subsequently treated for 30 min with 10% sodium thioglycolate to block tissue-derived sulfhydryl groups. After rinsing in 0.15 M sodium nitrate, sections were immersed twice for 20 min each in a mixture of solutions A and B of the IntenSE M silver Enhancement Kit (Amersham Biosciences, Little Chalfont, UK) for silver intensification of goldlabeled tracer molecules. Unspecifically tissue-bound silver ions were removed by fixation for 10 min in 5% sodium thiosulfate. After three

additional washes in 0.15 M acetate buffer, pH 5.6, silver deposits were stabilized by gold toning with 0.05% gold chloride in acetate buffer for 7 min in an ice bath.

1.4.10. Postembedding-immunogold cytochemistry at the electron microscopic level

For postembedding immunogold cytochemistry, ultrathin sections were pretreated with periodic acid and sodium metaperiodate [26]. After appropriate jet rinsing the grids were floated facing down on 50 µl drops containing the rabbit anti GABA antibody (Sigma-Aldrich Biochemie GmbH, Hamburg, Germany), diluted 1:1000 in 5% NGS, rinsed again, and treated with gold-labeled secondary antibody (1:50 in 2% NGS). Sections were washed, air dried, stained with uranyl acetate followed by lead citrate [25], and examined with a Zeiss EM912 electron microscope.

1.4.11. In situ hybridization

Riboprobe generation was described recently [27, 2]. Digoxigenin (DIG)-labeled antisense and sense probes were generated by linearizing plasmids with NotI (GAD65 and GAD67) and either SacII (GAD65) or SphI (GAD67) and by using the DIG RNA labeling Kit (Roche Diagnostics, Mannheim, Germany).

For in situ hybridization, freely floating sections were treated as described [28]. Sections were hybridized overnight at 56° C followed by stringency washes. After RNAse treatment the DIG-labeled RNA probes were visualized with anti-digoxigenin antibodies conjugated to alkaline phosphatase and the corresponding substrates nitroblue tetrazolium chloride and 5-bromo- 4-chloro-3-indolyl phosphate. Sections were mounted on gelatincoated slides, air-dried, and coverslipped under Vectamount (Vector Laboratories Inc, Burlingame, CA, USA).

1.4.12. Klüver-Barrera, Cresyl Violet and Methyl Green stain

Slide-mounted sections were left in 70% ethanol overnight, rinsed in bidistilled water, and stained either with cresyl violet (0.2% cresyl violet acetate in 20mM acetate buffer, pH 4.0) or methyl green (2% methyl green in 20mMacetate buffer, pH5.0) for 30 min at RT.

For the Klüver-Barrera staining freely floating sections were rinsed in PBS, mounted on gelatine-coated slides, followed by extraction of lipids in 70% ethanol for 12 hours as previously described [29, 30]. In short, myelinated fibers were stained overnight at 56°C in a 0.1% Luxol fast blue solution in 96% ethanol and 0.05% acetic acid. Thereafter, sections were washed in distilled water, differentiated for 3 minutes in 0.01% lithium carbonate and subsequent 3 minutes in 70% ethanol, washed in PBS and mounted on gelatine-coated slides. Subsequently, sections

were stained with cresyl violet as described earlier [30]. After rinsing in bidistilled water, sections were dehydrated and coverslipped.

1.4.13. Photographic documentation

Microscopic images were obtained with upright Leica DMRB light microscope connected to a high-resolution digital camera (Olympus SP-55UZ /Camedia C7070). Primary photographs were adjusted for brightness, color, and contrast in Adobe Photoshop CS3 (10.0) and arranged using Adobe Illustrator CS3 (13.0.0).

1.4.14. Digitization and data analysis

Somata of retrogradely labeled neurons were counted in sections regularly spaced at 250 μm along the anterior-posterior extent of the habenular [3] or VTA [1]. To assess overcounting error, we employed the Abercrombie correction method [31, 21]. Sections containing LHb or VTA were identified and visualized with Neurolucida 8.0 software (MBF Bioscience) through an upright Leica DMRB light microscope equipped with a high-resolution digital camera (MBFCX9000; MBF Bioscience), a mechanical stage (Ludl, Thornwood, NY), and an x–y–z axis encoder connected to a PC running Windows XP Professional. Each nucleus was outlined in tracing mode under 40-fold magnification. Neuronal somata that contained at least three distinctive tracer granules were counted and marked as ‘‘retrogradely labeled neurons.’’ Tagged maps of each section were converted into TIFF Images in Neurolucida Explorer.

1.5. Results

1.5.1. Glutamatergic axons from the lateral habenula mainly terminate on GABAergic neurons of the ventral midbrain

A first set of experiments aimed to analyze the neuronal connections from the LHb to the VTA including the corresponding transmitter systems. It is well known that electrical stimulation of the LHb results in a strong decrease of dopaminergic activity in the VTA [12, 13]. This effect could be due to a direct inhibitory, potentially GABAergic, input from the LHb targeting DA cells in the VTA. Alternately, incoming LHb axons may be excitatory and synapse on inhibitory neurons in the VTA. Our in-situ-hybridization experiments using RNA probes for GAD65 as well as GAD67 (Figure 1 in [2]) indicated that GABAergic neurons in the habenula are very scarce, leaving a direct inhibitory projection an unlike option. Close to all cells, however, displayed vGluT2 mRNA and protein (Figure 2 in [2]), suggesting an excitatory output from the LHb. This idea was confirmed by tracing experiments. For this purpose the retrograde tracer ApoHRP-WGA-gold was injected into the VTA. In-situ-hybridization and visualization of the retrogradely transported tracer molecules by silver intensification revealed black gold/silver granules in vGluT2-positive neurons (Figure 3 in [2]). This data provides evidence that the LHb indeed releases glutamatergic axons, which terminate in the VTA. Additional tracing experiments supported this conclusion. For this purpose the anterograde tracers (Phaseolus vulgaris leukoagglutinin, PhaL or TMR-DA) were injected into the LHb (Figure 4 in [2]). Tracer-positive axon terminals as well as potential transmitters (glutamate via vGluT2; GABA directly or via GAD67; dopamine via tyrosine hydroxylase, TH) were detected in the VTA by immunocytochemical double labeling on the electron microscopic level (Figure 6 & 7 in [2]). As it turned out, all tracer-labeled axons displayed vGluT2-immunoreactivity and therefore are glutamatergic (Figure 6 in [2]). Most (about 90%) of them target large GAD67-positive dendrites (Figure 7 in [2]), confirming the idea of an excitatory LHb input synapsing on inhibitory neurons in the VTA. There was, however, a much smaller number of labeled axons directly terminating on TH-positive dendrites (Figure 8 in [2]). Taking together, there are two distinct projections from the LHb to the VTA, potentially subserving different biological functions.

1.5.2. Anterior and posterior parts of the rat ventral tegmental area and the rostromedial tegmental nucleus receive topographically distinct afferents from the lateral habenular complex

The second set of experiments aimed to analyze in more detail the neuronal connections from the LHb to the anterior VTA (aVTA), the posterior VTA (pVTA) and the rostromedial tegmental nucleus (RMTg) (see figure below), which among other terms also is called tail or caudal pole of the VTA [32-34]. Hodological examination of the aVTA disclosed three distinct areas (Figure 3 & 4 in [3]), a dorsal layer and a ventral layer, which do not exactly correspond to the parabrachial pigmented or the paranigral subnucleus of the aVTA, and the rostral linear nucleus of the raphe (RLi).

Subsequent to injections of retrograde tracers (fluorogold, cholera toxoid, ApoHRP-WGA-gold) into the dorsal layer of the aVTA labeled neurons were found mostly ipsilaterally in the caudal half of the lateral division (LHbL) of the LHb (Figure 4 left in [3]). In contrast, tracer application into the ventral layer yielded positive cells throughout the longitudinal axis of the LHb, predominantly ipsilaterally and favoring the medial division (LHbM) of the LHb (Figure 4 right in [3]). Tracer injections, which were largely restricted to the RLi, disclosed that this subfield receives bilateral projections from the LHb. Interestingly, labeled neurons were concentrated in the area of the oval subnucleus of the LHbL (Figure 3 in [3]). This subnucleus appears to be largely avoided by the other connections.

In contrast to the aVTA there are no obvious hodological differences between layers in the pVTA. Consequently, four distinct areas, the paranigral, the parabrachial pigmented, and the caudal linear subfields of the VTA and in addition the RMTg were chosen for selective injections. In general these areas received a largely bilateral input from the LHb. Projecting neurons to the paranigral subfield of the pVTA were distributed over most of the LHb, preferring the LHbM and especially its superior subnucleus and avoiding the oval subnucleus of the LHbL (Figure 5 in [3]). In contrast, connections to the parabrachial pigmented and the RMTg preferentially originated in the LHbL. The oval subnucleus of the LHbL was again largly free from cells targeting the RMTg (Figure 6 left in [3]). A distinct group of cells releasing axons to the parabrachial pigmented subnucleus was centered in the parvocellular subnucleus of the LHbL (Figure 6 right in [3]). A very similar group of neurons is prominently labeled subsequent to injections into the caudal linear nucleus (CLi) of the pVTA (Figure 7e in [3]). Most likely, this cell group predominantly projects to the parabrachial pigmented subfield of the pVTA and its appearance after injections into the CLi may be due to some spread of the tracer in the neighboring area.

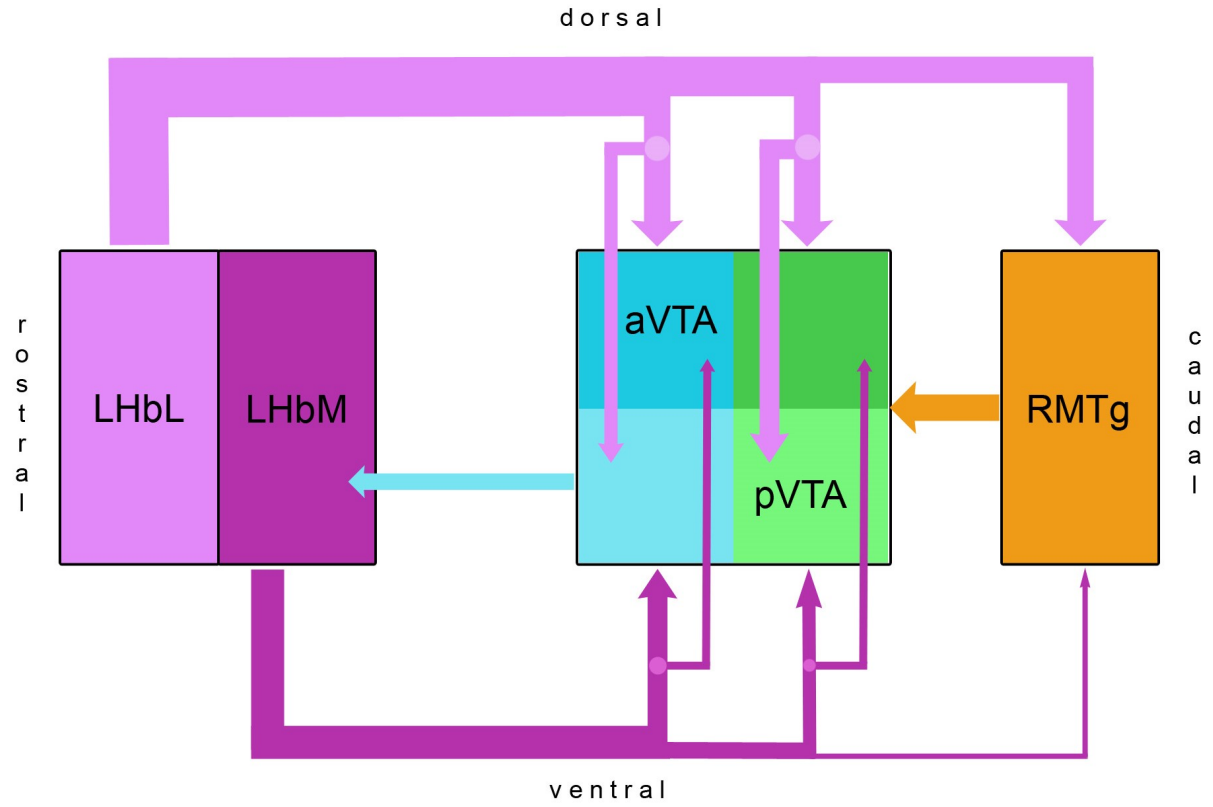

Figure: Schematic representation of the projections from the lateral habenula to its target regions

Projections from the lateral LHb (LHbL) to the VTA and the rostromedial tegmental nucleus (RMTg) are stronger than those from the medial LHb (LHbM). The LHbL sends major projections directly to the dorsal layer of the anterior VTA (aVTA) and posterior VTA (pVTA) and indirectly via the RMTg to the VTA. LHbM neurons, in contrast, project to the ventral layer of aVTA and to a smaller extend to the paranigral subnucleus of the pVTA. LHbM output to the RMTg is sparse. LHb axons target predominantly ipsilateral cells in the aVTA, and bilateral in the pVTA and RMTg; as detailed in figure 9 in [3].
The dopaminergic back projection from VTA to LHb mainly has its origin in the ventral layer of the aVTA (paramedian field) and targets the LHbM. Dorsal layer of VTA is presented in dark blue (aVTA) and dark green (pVTA), ventral layer of VTA subareas shown in light blue (aVTA) and light green (pVTA).

There are, however, other cell groups, which do not show up after injection into the PBP and may contain neurons selectively projecting to the CLi (Figure 7c, d in [3]).

Retrograde tracing data was confirmed by anterograde tracing experiments. Connections originating in the medial LHb yielded axon terminals mostly ipsilaterally in the PN of the aVTA and bilaterally in the pVTA (Figure 8 left in [3]), while fibers from the lateral LHb targeted the PBP bilaterally along its complete rostrocaudal axis (Figure 8 right in [3]). Anterograde tracer injections into the LHbL unevitably included the LHbLO. Consequently, the corresponding terminals were clearly evident in the RLi area (Figure 8 right in [3]). For a schematic representation of the LHb-VTA-RMTg axis see figure above.

1.5.3. The back projection from the VTA to the LHb is stronger as suggested by literature data

Facing the fact that the lateral habenular complex is a center of negative reward, a detailed understanding of the dopaminergic back projection from the VTA to the LHb is important. In fact, LHb activity is modulated by locally released dopamine [35, 36], which is largely derived from the ventral tegmental area via the mesohabenular pathway [37]. In contrast to the prominent effect of dopamine in the LHb, the number of dopaminergic axons from the VTA to the LHb was considered rather low, between 1% and 10% of all mesohabenular fibers [37, 38].

Consequently, in our study we used contemporary techniques to reinvestigate number and origin of dopaminergic fibers from the VTA to the LHb. Therefore a retrograde tract-tracer gold-coupled wheat germ agglutinin was injected into the LHb of rats. By far most of the retrogradely neurons were found in the anterior VTA, accumulating in its ventral paramedian fields (see figure, blue arrow). About 47% of labeled cells displayed tyrosine hydroxylase immunoreactivity, suggesting that almost half of the mesohabenular neurons are dopaminergic. To our surprise we found that incerto-hypothalamic and periventricular neurons also contribute dopaminergic terminals to the LHb. The majority of dopaminergic axons terminate in the LHbM (see figure, blue arrow). The LHbLO apparently plays an unconventional role in the LHb network. It rather selectively projects to the RLi, is largely devoid of axons originating in the aVTA or the RMTg, and hardly contains any dopaminergic terminals.

1.6. Discussion

1.6.1. Methodological considerations

The present investigation largely uses anterograde and retrograde tracing techniques to increase our understanding of the LHb-VTA network. Inherent difficulties with tracing experiments include the accuracy of the tracer injection, tracer spread into adjacent areas, and the damage of passing fibers during injection. In addition, it is often important to know the transmitter phenotype of the traced neurons or axons. Quite often regions of interest were very small, precluding an injection restricted within the borders of the target area. This problem was largely solved by summing up several similar injections, by comparing the results of injections into adjacent areas and by the combined analysis with anterograde and retrograde tracers.

In principle, tracing techniques allow to visualize labeled neurons and axons in quite a detail without any information concerning the transmitter phenotype of the corresponding neuron or its targets. In the present investigation, therefore, double immunocytochemistry at the light and electron microscopic level was used to simultaneously visualize the tracer in combination with an appropriate marker for the corresponding transmitter. In addition, rats, but not mice were used as experimental model animals, as most information on the connectivities of LHb and VTA is available in the rat. Furthermore, subnuclei usually are small even in the rat, and still smaller in the mouse, making selective injections rather difficult.

1.6.2. Are the connections between the LHb and the VTA reciprocal?

Reciprocal connections between distinct brain areas are often described, but seldom understood precisely. Unfortunately, the term “reciprocal” is ambiguous. As explained in detail recently [3] it offers different interpretations. “Does it just mean that a given brain area projects to another one and gets recurrent connections from there? In this case the biological meaning of the circuit is not directly obvious. However, “reciprocal” could also mean that cortical laminae, columns, or subcortical nuclei project to other nuclei and receive a return projection from there. Or, even more specific, it may imply that an efferent neuron anywhere is contacted by axons originating from its target neurons. Is this actually the case in one of the most renowned “reciprocal” connections, the thalamocortical?

In this loop, neurons of palliothalamic nuclei (core-type relay neurons; [39]) largely project to cortical layer 4, where they target the main dendrites of lamina 5 pyramidal tract type neurons (PT neurons) so intensely that they are able do drive these neurons even without additional inputs from other local excitatory cells [39]. PT neurons, however, mostly project to distant

subcortical structures like the spinal cord with minor collaterals to the thalamus. Major cortical projections to the thalamus, in contrast, arise from layer 6 corticothalamic neurons (CT; [39]). Thus, when analyzed in detail, even the thalamocortical-corticothalamic loop is not precisely a reciprocal one.

The habenula often is considered as relay station for reciprocal transport of information between limbic and midbrain areas [1, 40-46]. In fact, there may be more than one reciprocal connection between the LHb and the VTA. First, our present data indicate that the habenular input to the RLi originates from the LHbL, especially in the LHbLO (see Figure 3 in [3]). Complementary injections of the anterograde tracer PhaL into the RLi result in labeled axon terminals predominantly in the LHbLO [47]. Thus, the connection between the RLi and the LHbLO is reciprocal in the strict sense, indeed, if not at the neuronal, then at least at the subnuclear level. Second, our present data indicate that the habenular input to the dorsal layer of the aVTA mainly originates from the ipsilateral LHbL. In turn, efferent projections from the dorsal aVTA to habenula are known to target the LHbL area [47, 48]. Again, the connectivity appears to be reciprocal. Third, our data indicate that the habenular input to the ventral layer of the aVTA mainly originates from the LHbM (see Figure 4, right side in [3]). In turn, efferent projections from the ventral aVTA are known to target the LHbM [1, 43, 47]. Thus, the connection between the ventral aVTA and the LHbM again appears reciprocal as had been suggested earlier [43]."

1.6.3. Two separate pathways from the LHb to the VTA, a direct and an indirect one, may be necessary to subserve distinct biological functions

The idea of the LHb as an anti-reward center is at least partially based on the direct and in fact inhibitory projection from the LHb to the VTA. A few years ago the RMTg was newly described, which receives excitatory input from the LHb and sends inhibitory axons to the VTA. Since then, a considerable group of investigators tended to ignore the direct projection from the LHb to the VTA, even in spite of the fact that LHb neurons projecting to the VTA and those targeting the RMTg are largely non overlapping populations [43, 49]. Our present investigations emphasize the presence of two pathways from the LHb, a direct one, which may be involved in the process of anhedonia, and an indirect one, which may be part of freezing behavior.

In opposite to reflexes behavioral action requires a complex computational network that integrates and analyzes many inputs to select the appropriate action or response. While we acknowledge the enormous complexity of brain connectivity, in communicating there is often tendency to reduce or simplify for better understanding. This is true for the relationship between habenula and VTA which is often reduced to a one-directional-pathway and involves the RMTg

as necessary relay. While such pathway exists and is connected with one behavioral response, the relationship between VTA and habenula, their structural connectivity and resulting behavioral phenotypes are much more widespread. Meticulous neuronal tracing is a helpful technique to discover and decipher existing neuronal connections. Therefore, the present work has elucidated several direct, subnuclear-specific connections between both reward-related midbrain structures that are distinct and reciprocal. While the exact behavioral paradigm for these networks is not fully understood yet, the present findings offer plentiful new testable hypotheses.

1.7. References

1 Gruber C, Kahl A, Lebenheim L, Kowski A, Dittgen A, Veh RW. 2007. Dopaminergic projections from the VTA substantially contribute to the mesohabenular pathway in the rat. Neurosci Lett 427:165-170.

2 Brinschwitz K, Dittgen A, Madai VI, Lommel R, Geisler S, Veh RW. 2010. Glutamatergic axons from the lateral habenula mainly terminate on GABAergic neurons of the ventral midbrain. Neurosci 168:463-476.

3 Petzel A, Bernard B, Poller WC, Veh RW. 2017. Anterior and posterior parts of the rat ventral tegmental area and the rostromedial tegmental nucleus receive topographically distinct afferents from the lateral habenular complex. J Comp Neurol 525(10):2310-2327.

4 Wagner F, Weiss T, Veh RW. 2017. Electrophysiological Properties of Neurons and Synapses in the Lateral Habenular Complex (LHb). Pharmacol Biochem Behav. In revision.

5 Wagner F, Bernard R, Derst D, French L, Veh RW. 2016b. Microarray analysis of transcripts with elevated expressionsin the rat medial or lateral habenula suggest fast GABAergic excitation in the medial habenula and habenular involvement in the regulation of feeding and energy balance. Brain Struct Funct 221:4663-4689.

6 Bear MF, Connors BW, Paradiso MA. 2007. Neuroscience: Exploring the Brain. Baltimore: Williams & Wilkins.

7 Tortora GJ. 2004. Principles of Human Anatomy. 10th Edititon: John Wiley & Sons, Inc.

8 Schultz W. 1998. Predictive reward signal of dopamine neurons. J Neurophys 80:1-27.

9 Wise R. 2004. Dopamine, learning and motivation. Nature Rev 5:1-12.

10 Kringelbach ML, Berridge KC. 2010. The Neuroscience of Happiness and Pleasure. Soc Res (New York) 77:659–678.

11 Amo R, Aizawa H, Takahoko M, Kobayashi M, Takahashi R, Aoki T, Okamoto H. 2010. Identification of the zebrafish ventral habenula as a homolog of the mammalian lateral habenula. J Neurosci.; 30(4):1566-74.

12 Christoph GR, Leonzio RJ, Wilcox KS. 1986. Stimulation of the lateral habenula inhibits dopamine-containing neurons in the substantia nigra and ventral tegmentum area of the rat. J Neurosci 6:613-619.

13 Ji H, Shepard PD. 2007. Lateral Habenula Stimulation Inhibits Rat Midbrain Dopamine Neurons through a GABA-A Receptor-Mediated Mechanism. J Neurosci 27:6923-6930.

14 Matsumoto M, Hikosaka O. 2007. Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 447:1111-1115.

15 Chastrette N, Pfaff DW, Gibbs RB. 1991. Effects of daytime and nighttime stress on Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus. Brain Res 563:339-344.

16 Ullsperger M, von Cramon DY. 2003. Error monitoring using external feedback: Specific roles of the habenular complex, the reward system, and the cingulate motor area revealed by functional magnetic resonance imaging. J Neurosci 23:4308-4314.

17 Shepard PD, Holcomb HH, Gold JM. 2006. The presence of absence: Habenular regulation of dopamine neurons and the encoding of negative outcomes. Schizophrenia Bull 32:417-421.

18 Kringelbach ML. 2005. The human orbitofrontal cortex: Linking reward to hedonic experience. Nat Rev Neurosci 6:691-702.

19 Kowski AB. 2006. Die Strukturelle Komplexität des lateralen Habenularkomplexes und lateral preoptisch - hypothalamischen Region im Vergleich mit hodologischen Daten.

20 Poller WC, Madaia VI, Bernard R, Laube G, Veh RW. 2013. A glutamatergic projection from the lateral hypothalamus targets VTA-projecting neurons in the lateral habenula of the rat. Brain Res 1507:45–60.

21 Kowski A, Geisler S, Krauss M, Veh RW. 2008. Differential Projections from Subfields in the Lateral Preoptic Area to the Lateral Habenular Complex of the Rat. J Comp Neurol 507:1465–1478.

22 Paxinos G, Watson C. 1998. The Rat Brain in Stereotaxic Coordinates. San Diego: Academic Press Ltd.

23 Somogyi P, Takagi H. 1982. A note on the use of picric acid-paraformaldehyde-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. J Neurosc. 7(7):1779-83.

24 Andres KH, von Düring M. 1981. General methods for characterization of brain regions. In: Forssmann CHWG, editor. Techniques in Neuroanatomical Research. Heidelberg: Springer. p 100-108.

25 Reynolds ES. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208-212.

26 Ottersen OP. 1987. Postembedding light- and electron microscopic immunocytochemistry of amino acids: description of a new model system allowing identical conditions for specificity testing and tissue processing. Exp Brain Res 69:167-174.

27 Geisler S, Derst C, Veh RW,Zahm DS. 2007. Glutamatergic Afferents of the Ventral Tegmental Area in the Rat. J Neurosci 27:5730-5743.

28 Wagner F, French L, Veh RW. 2016a. Transcriptomic-anatomic analysis of the mouse habenula uncovers a high molecular heterogeneity among neurons in the lateral complex, while gene expression in the medial complex largely obeys subnuclear boundaries. Brain Struct Funct 221:39-58.

29 Klüver H, Barrera E. 1953. A method for the combined staining of cells and fibers in the nervous system. J Neuropath Exp Neurol 12:400-403.

30 Geisler S, Heilmann H, Veh RW. 2002. An optimized method for simultaneous demonstration of neurons and myelinated fiber tracts for delineation of individual trunco- and palliothalamic nuclei in the mammalian brain. Histochemistry and Cell Biology 117:69-79.

31 Abercrombie M. 1946. Estimation of nuclear population from microtome sections. Anat Rec 94:239-247.

32 Ferreira JGP, Del-Fava F, Hasue RH, Shammah-Lagnado SJ. 2008. Organization of ventral tegmental area projections to the ventral tegmental area-nigral complex in the rat. Neurosci 153:196-213.

33 Kaufling J, Veinante P, Pawlowski SA, Freund-Mercier M-J, Barrot M. 2009. Afferents to the GABAergic Tail of the Ventral Tegmental Area in the Rat. J Comp Neurol 513:597-621.

34 Jhou TC, Fields HL, Baxter MG, Saper CB, Holland PC. 2009b. The rostromedial tegmental nucleus (RMTg), a major GABAergic afferent to midbrain dopamine neurons, selectively encodes aversive stimuli and promotes behavioral inhibition. Neuron 61:786–800.

35 McCulloch J, Savaki HE, Sokoloff L. 1980. Influence of dopaminergic systems on the lateral habenular nucleus of the rat. Brain Res 194:117-124.

36 Kowski AB, Veh RW, Weiss T. 2009. Dopaminergic activation excites rat lateral habenular neurons in vivo. Neurosci 161:1154-1165.

37 Skagerberg G, Lindvall O, Björklund A. 1984. Origin, Course and Termination of the Mesohabenular Dopamine Pathway in the Rat. Brain Res 307:99-108.

38 Swanson LW. 1982. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9:321-353.

39 Harris KD, Shepherd GMG. 2015. The neocortical circuit: themes and variations. Nature Neurosci 18:170-181.

40 Herkenham M, Nauta WJH. 1979. Efferent Connections of the Habenular Nuclei in the Rat. J Comp Neurol 187:19-48.

41 Sutherland RJ. 1982. The Dorsal Diencephalic Conduction System: A Review of the Anatomy and Functions of the Habenular Complex. Neurosci Biobehav Rev 6:1-13.

42 Araki M, McGeer PL, Kimura H. 1988. The efferent projections of the rat lateral habenular nucleus revealed by the PHA-L anterograde tracing method. Brain Res 441:319-330.

43 Goncalves L, Sego C, Metzger M. 2012. Differential Projections From the Lateral Habenula to the Rostromedial Tegmental Nucleus and Ventral Tegmental Area in the Rat. J Comp Neurol 520:1278–1300.

44 Shen X, Ruan X, Zhao H. 2012. Stimulation of Midbrain Dopaminergic Structures Modifies Firing Rates of Rat Lateral Habenula Neurons. PLoS ONE 7:e34323.

45 Stamatakis AM, Jennings JH, Ung RL, Blair GA, Weinberg RJ, Neve RL, Boyce F, Mattis J, Ramakrishnan C, Deisseroth K, Stuber GD. 2013. A Unique Population of Ventral Tegmental Area Neurons Inhibits the Lateral Habenula to Promote Reward. Neuron 80:1039-1053.

46 Zuo W, Zhang Y, Xie G, Gregor D, Bekker A, Ye J-H. 2016. Serotonin stimulates lateral habenula via activation of the postsynaptic serotonin 2/3 receptors and transient receptor potential channels. Neuropharmacol 101:449-459.

47 Del-Fava F, Hasue RH, Ferreira JGP, Shammah-Lagnado SJ. 2007. Efferent connections of the rostral linear nucleus of the ventral tegmental area in the rat. Neurosci 145:1059-1076.

48 Beckstead RM, Domesick VB, Nauta WJH. 1979. Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res 175:191-217.

49 Li B, Piriz J, Mirrione M, Chung C-H, Proulx CD, Schulz D, Henn F, Malinow R. 2011. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. Nature 470:535-539.

2. Eidesstattliche Versicherung & Anteilserklärung

„Ich, Anja Petzel, geborene Dittgen, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema “The Mammalian Lateral Habenular Complex – Projection and Back Projection to the Ventral Tegmental Area, the Center of the Reward System“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -www.icmje.org) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.
Meine Anteile an den ausgewählten Publikationen entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem Betreuer, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.
Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

_____

Berlin, 22.06.2017 Anja Petzel

Anteilserklärung an den erfolgten Publikationen

Anja Petzel (geb. Dittgen) hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: [Gruber C, Kahl A, Lebenheim L, Kowski A, **Dittgen A**, Veh RW], [Dopaminergic projections from the VTA substantially contribute to the mesohabenular pathway in the rat] [Neurosci Lett], [2007]

Beitrag im Einzelnen: A.P. leistete einen wesentlichen Anteil an der Konzeption und Durchführung der Versuche, des Neurolucida-Plottings und Auszählens und bei Erstellung des Manuskripts.

Publikation 2: [Brinschwitz K, **Dittgen A**, Madai VI, Lommel R, Geisler S, Veh RW], [Glutamatergic axons from the lateral habenula mainly terminate on GABAergic neurons of the ventral midbrain], [Neuroscience], [2010]
Beitrag im Einzelnen: A.P. leistete einen wesentlichen Beitrag an der Zusammenstellung und Durchführung der Tracing-Versuche, WGA-gekoppelten In-Situ-Hybridisierung und der Färbungen. Außerdem war sie maßgeblich an der Erstellung des Manuskripts und der Grafiken beteiligt.

Publikation 3: [**Petzel A**, Bernard B, Poller WC, Veh RW], [Anterior and posterior parts of the rat ventral tegmental area and the rostromedial tegmental nucleus receive topographically distinct afferents from the lateral habenular complex], [J Comp Neurol], [2017]
Beitrag im Einzelnen: A.P. leistete einen wesentlichen Beitrag bei der Konzeption, der Zusammenstellung und Durchführung der Versuche, der Analyse und Auswertung der Experimente, Literaturrecherche und Erstellung des Manuskripts und der Grafiken.

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Berlin, 22.06.2017 Prof. em. Rüdiger W. Veh

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Berlin, 22.06.2017 Anja Petzel

3. Published Manuscripts

3.1. "Dopaminergic projections from the VTA substantially contribute to the mesohabneular pathway in the rat"

3.2. "Glutamatergic axons from the lateral habenular mainly terminate on GABAergic neurons of the ventral midbrain"

3.3. "Anterior and posterior parts of the rat ventral tegmental area and the rostromedial tegmental nucleus receive topographically distinct afferents from the lateral habenular complex"

Gruber C, Kahl A, Lebenheim L, Kowski A, Dittgen A, Veh RW. 2007. Dopaminergic projections from the VTA substantially contribute to the mesohabenular pathway in the rat. Neurosci Lett 427:165-170.

DOI: https://doi.org/10.1016/j.neulet.2007.09.016

Brinschwitz K, Dittgen A, Madai VI, Lommel R, Geisler S, Veh RW. 2010. Glutamatergic axons from the lateral habenula mainly terminate on GABAergic neurons of the ventral midbrain. Neurosci 168:463-476.

DOI: https://doi.org/10.1016/j.neuroscience.2010.03.050

Petzel A, Bernard B, Poller WC, Veh RW. 2017. Anterior and posterior parts of the rat ventral tegmental area and the rostromedial tegmental nucleus receive topographically distinct afferents from the lateral habenular complex. J Comp Neurol 525(10):2310-2327.

DOI: https://doi.org/10.1002/cne.24200

4. Curriculum vitae

Anja Petzel

"Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht."

"Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht."

Berlin, 22.06.2017 Anja Petzel

5. Bibliography

5.1.Publications

Petzel A, Bernard B, Poller WC, Veh RW. 2017. Anterior and posterior parts of the rat ventral tegmental area and the rostromedialtegmental nucleus receive topographically distinct afferents from the lateral habenular complex. J Comp Neurol 525(10):2310-2327.

Brinschwitz K, **Dittgen A,** Madai VI, Lommel R, Geisler S, Veh RW. 2010. Glutamatergic axons from the lateral habenula mainly terminate on GABAergic neurons of the ventral midbrain. Neurosci 168:463-476.

Favero G, **Dittgen A**, Pfiffer T, Köhler C, Schneider A. Zervixkarzinom in derSchwangerschaft. 2010. Frauenarzt 51, Nr. 2.

Gruber C, Kahl A, Lebenheim L, Kowski A, **Dittgen A,** Veh RW. 2007. Dopaminergic projections from the VTA substantially contribute to the mesohabenular pathway in the rat. Neurosci Lett 427:165-170.

5.2.Poster

Petzel A, Fischer T, Slowinski T, Thomas A.
Die ZervixelastographiealsPrädiktorderGeburtsdauerbeiGeburtseinleitung.
26. DeutscherKongressfürPerinataleMedizin, Posterpräsentation (2013)

Petzel A, Fischer T, Slowinski T, Thomas A.
KannmitHilfederZervixelastographieeinePrädiktionderGeburtsdauererfolgen?
25. DeutscherKongressfürPerinataleMedizin, Posterpräsentation (2011)

Dittgen A, Gruber C, Geisler S, Zahm DS, Veh RW.2006. Anterior and posterior parts of the rat ventral tegmental area receive topographically distinct afferents from the lateral habenular complex. Soc NeurosciAbstr.; 670.24/LL33

6. Danksagung

Danke – an Rüdiger W. Veh – für die Liebe zur Anatomie, ein spannendes Dissertationsthema, wissenschaftliches Handwerkszeug, die Vermittlung fundierter Forschung, für seine Geduld und Zuversicht und die stete Unterstützung bis zum Abschluss dieses Projekts. Rüdiger war und ist ein Mentor und wichtiger Berater in vielen Lebenslagen.

Danke – an René Bernard – für seine Freundschaft, die hilfreichen inhaltlichen und sprachlichen Anmerkungen. René war ein unerschütterlicher Begleiter so vieler „letzter" Anläufe, der nie müde wurde zu betonen, ich würde es schaffen.

Danke – an Annett Kaphahn, Ina Wolter und Heike Heilmann – die guten Seelen der Arbeitsgruppe, bei denen man sich immer Willkommen fühlte, ein offenes Ohr und einen Cappuccino fand. Sie standen allzeit mit Rat und Tat unterstützend zur Seite.

Danke – an Katja, die „Brinsch", – meine gute Freundin und Mitstreiterin an vielen Abenden im Labor. Nicht selten hat sie mit mir, der „Frau von unten", die wissenschaftlichen Diskussionen bei Baguette und Rotwein auf dem Balkon fortgesetzt.

Danke – an Tina Kienitz – meine Schwester im Geiste. So viel hast du für mich getan und mich unermüdlich unterstützt bis zum Schluss. Schön, dass es dich gibt. Ich hoffe, in eurem nächsten Großprojekt kann ich dir auch eine Stütze sein.

Danke – meinen Eltern – für Ihre Liebe und gut gemeinten Nachfragen, was denn die Promotion so macht. Es tut mir leid, wenn ich bisweilen etwas ruppig reagierte. Eure Anteilnahme schätze ich sehr.

Danke – meinem Mann Marc und meinen beiden Mäusen Linus und Louisa – mein Antrieb, mein sicherer Hafen und Lebensmittelpunkt. Ich liebe euch.

"Das Höchste, wozu der Mensch gelangen kann, ist das Erstaunen."

Johann Wolfgang von Goethe