From DEPARTMENT OF NEUROSCIENCE
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ON INNATE BEHAVIORS: FOCUS ON PARENTAL BEHAVIOR AND AGGRESSION

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On the cover: This acrylic paint and spray art piece strives to encompass the observations and feelings conveyed by the scientific endeavors contained in this thesis, as perceived by the artist.

The rounded red and black orbs signify an overarching and omnipresent possibility for aggression. Their curved forms representative of the spiritual, they are however bound within the confines of a rectangle illustrative of the physical boundaries of the skull. Five paths of differing lengths span over this aggressive mindscape. They are individual paths, all sacred as the golden ratio that connects them to each other. These paths do not branch however, as a sense of determinism emanates from the observations of this thesis. An ancient symbol of dominance and hierarchy, the pyramid, is ubiquitously found over each path. They are all the same pyramid, but they all take slightly different colors to represent a nearly comical repetition of the same behavior in different individuals. Rainwater from a storm was collected and was used to clean the brushes in between brushstrokes. This was done in the hopes of infusing into the painting the essence of the storm, another classic representation of divine wrath. Painted with a brusque, neo-expressionist style in mind, the paths and the pyramids are also a resentful, rebellious outlook on the predetermined unravelling of emotions and behaviors throughout life.

Credit: Nicolas Scalbert

On innate behaviors:
focus on parental behavior and aggression

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To my parents.

ABSTRACT

The neural substrates orchestrating a number of social behaviors, including parental behavior and aggression, are known to exist in the hypothalamus. Through the control of the pituitary gland, the hypothalamus regulates the release of a number of hormones necessary for the physiological control of bodily functions and the expression of appropriate behaviors. In recent years the neuroscience community has invested large resources in identifying, through molecular markers, subsets of neurons whose activity impacts behavioral expression. This approach, however, has several weaknesses, among which is the assumption that a neuron's function and output adhere to generalized principles. Consequently, such investigations often fail to identify the intricate organization of neural networks, which adapt the neural code in order to tune a system's output to the behavior it modulates.

The aim of this thesis is to expand on basic neurophysiological concepts regarding the complex organization within and among neural groups. Here we addressed the principles of how a set of neurons self-tune their activity through the use of their own neurotransmitter, intra- and inter-network connectivity designs and spike rate coding of neurotransmitter release. Following this interrogation of neural network properties, we attempted to link the activity of these neural nodes to behavioral output, where we identified two distinct subsets of neurons driving parental behavior and aggression respectively.

In paper I, we performed a study on the properties of autoregulation in a neural network, and identified the ionic mechanisms through which the tuberoinfundibular dopamine (TIDA) neurons control their own activity via the use of their own neurotransmitter, dopamine (DA). In paper II, we encountered an unexpected species difference in baseline activity and oscillation frequency between rat and mouse TIDA neurons. Following an in-depth investigation, we attributed this difference to the presence *vs* complete absence of electrical coupling in the rat and mouse TIDA cells respectively. This generated the question of how different modes of TIDA neuron activity impact DA release at their terminals, which was addressed in paper III where, using fast-scan cyclic voltammetry, we performed the first investigation coupling patterns of electrophysiological activity to DA release in the TIDA system. In paper IV we addressed the possibility that this discrepancy in TIDA neuron activity has a behavioral impact. Following a step-by-step breakdown of the lactotropic axis in the male rat and mouse, we ultimately provided a link between TIDA neuron activity and the suppression *vs* expression of paternal behavior in the two species.

The final part of this thesis includes two studies focusing on aggressive behavior. In paper V, we performed a functional interrogation of a subset of ventral premammillary (PMv) neurons involved in intermale aggression, while in paper VI we identified that the very same neurons are activated by maternal hormones and modulate the expression of maternal aggression in lactating female mice.

Overall, the work presented in this thesis provides a step forward in our understanding of neural function and on the neural substrates underlying social behavior.

LIST OF SCIENTIFIC PAPERS

I. **Stagkourakis S**, Kim H, Lyons DJ, Broberger C
   Dopamine Autoreceptor Regulation of a Hypothalamic Dopaminergic Network.
   Cell reports, April 14th 2016, 15: 735-747

II. **Stagkourakis S**, Pérez CT, Hellysaz A, Ammari R, Broberger C
   Network oscillation rules imposed by species-specific electrical coupling.
   eLife, May 3rd 2018, 7: 1-18

III. **Stagkourakis S**, Dunevall J, Taleat Z, Ewing A, Broberger C
   Dopamine release dynamics in the tuberoinfundibular dopamine system.
   Manuscript

IV. **Stagkourakis S**, Williams P, Kakadellis S, Ziegler K, Bakker J, Harkany T, Broberger C
   Neurohormonal basis of paternal behavior.
   Manuscript

V. **Stagkourakis S**, Spigolon G, Williams P, Protzmann J, Fisone G, Broberger C
   A neural network for intermale aggression to establish social hierarchy.
   Nature Neuroscience, May 25th 2018, 21: 834-842

VI. **Stagkourakis S**, Williams P, Spigolon G, Ziegler K, Heikkinen L, Fisone G, Broberger C
   Control of maternal aggression via maternal-hormone sensitive hypothalamic neurons.
   Manuscript

CONTENTS

LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
AP	Action potential
ARCdm	Dorsomedial arcuate nucleus
Cx36	Connexin 36
DA	Dopamine
DAT	Dopamine transporter
FSCV	Fast-scan cyclic voltammetry
ME	Median eminence
MPOA	Medial preoptic area
PMv	Ventral premammillary nucleus
PMv^{DAT}	DAT positive neurons in the ventral premammillary nucleus
Prl	Prolactin
PRT	Pup retrieval test
TIDA	Tuberoinfundibular dopamine

1 INTRODUCTION

One of the most challenging and beautiful quests to be pursued in science is the development of an understanding of the neural substrates driving behavior. From the flick of a tail in the escape response of a fish, to the battling of stags during mating season, animal behavior has motivated scientists over generations to investigate its origins. Darwin's theory of evolution offered a foundation stone in interpreting behavior, and a universal concept to explain the expression and presence of behavioral traits in individuals of a species[1-3]. An intriguing, analogous goal in the field of neuroscience is the formulation of a theory which can globally explain the information coding mechanisms utilized by neurons, allowing appropriate output of diverse systems engaged in radically different functions.

This will likely require an advance in knowledge across neuroscience sub-disciplines, including neurophysiological, anatomical, computational, and functional investigations of network activity *in vitro* and *in vivo*. The work in this thesis is an attempt to push the boundaries in that direction, looking at the neural substrates of innate behaviors.

1.1 PRINCIPLES OF NEURAL FUNCTION

Sir Charles Sherrington is considered the most prolific pioneer during the early development of the neuroscience field which, through his work, advanced both conceptually and methodologically. His landmark studies performed in the dawn of the 20th century include those on spinal cord reflexes[4-7] and the corticospinal projection[8-11], which raised the concepts of motor units, flexor-extensor reflex and the law of reciprocal innervation – concepts deeply embedded in today's neuroscience thinking. Following this, the classical work pursued by Golgi and Cajal and the formulation of the neuron doctrine[12-14] laid down the foundation of the neuroscience field. Subsequent original work in the years between 1940 and 1960 defined the basic mechanistic principles of neural function.

Initial work identified that the main currency for communication in the nervous system, the action potential (AP), is associated with an increase in membrane conductance[15], while in 1939, Hodgkin and Huxley performed the first recordings of APs from inside a nerve fiber. Work from John Eccles was instrumental for the development of an understanding of the AP and synaptic potentials in the nervous system[16-19]. Following this, the properties and ionic mechanisms governing membrane and action potentials, were investigated in depth[20-27]. Around the same time, Bernard Katz suggested the quantal theory of neurotransmitter release, a principle determining the post-synaptic changes in membrane potential[28, 29], while the cable theory suggested by Wilfrid Rall, corrected previous estimates of the conduction dynamics governing neural function[30-32].

From a computational perspective, the pioneering work of McCullock and Pitts in 1943 suggested the mechanisms through which neurons could connect together to form networks, leading to a meaningful system outcome[33, 34]. Though a simplified version of a biological

neuron, the McCulloch and Pitts neuron model (MCP neuron) - composed of a preprocessing unit (dendrite), and a processing/commanding unit (the soma) - identified the importance of a computational approach for the development of an understanding of the nervous system.

The identification of these principles facilitated an elaborate understanding of the neuron and the pursuit of questions at the neural network level. Yet the circuit organization of a system poses many additional challenges on information coding and processing, and among the first challenges that a neural network is required to solve is to develop mechanisms of self-control, restricting itself from entering inescapable states of quiescence or runaway excitation.

1.2 AUTOREGULATION IN NEURAL SYSTEMS

Autoregulation is an essential and widespread property found in most biological systems at many levels, including the genetic[35, 36], neural[37-44] and organ[45-47] levels.

Perhaps it is no surprise that, like in other systems, neural networks employ mechanisms to control their own activity[48-51]. Such an example can be found in the raphe nuclei, where the serotonin neuron autoregulation properties have been extensively investigated in the hope of identifying viable means of pharmacologically modulating their activity for the treatment of mood disorders[38]. For similar reasons, the mechanisms underlying autoregulation of the midbrain DA neurons have been in the spotlight of neuroscience research[52-55].

Here, in Paper I, we investigated the autoregulatory properties of the rat tuberoinfundibular dopamine (TIDA) neurons in the dorsomedial arcuate nucleus (ARCdm), which exhibit a synchronous, robust slow oscillation[56]. Following the study on TIDA neuron autoregulation, their oscillation properties attracted our interest. Such phenomena often rely on gap junction coupling, and we next pursued the role of gap junctions in this system.

1.3 GAP JUNCTIONS AND OSCILLATION FREQUENCY

Communication between neurons occurs in the nervous system in anatomically identifiable regions called synapses. The two major modalities mediating synaptic transmission are the chemical and the electrical synapses[57]. While chemical synapses use a mediator (a neurotransmitter or neuropeptide) to induce postsynaptic voltage changes, the electrical synapses allow charge to flow through specialized membrane pores, known as connexons (the functional unit of an electrical synapse). Both synapses are subject to elaborate forms of modulation, which can facilitate or depress the amplitude of the postsynaptic effect[57]. The electrical synapse, also known as a gap junction, is a ubiquitous method of intercellular communication found in the nervous system of both vertebrate and invertebrate animals[58-67]. Gap junction properties include high speed, bidirectional and reliable communication between neurons, while a single gap junction is able to both inhibit and excite a postjunctional target. These features represent distinct advantages of gap junctions over chemical synapses[68-73].

2

Importantly, electrical synapses offer key features in neural networks including phase locking of action potential firing or membrane potential fluctuations[74-80]. It is in the formation of the gap junction, and the connexin protein subtypes which come together to form the connexon, that the properties of the electrical synapse are defined[69, 81-85]. Cellular location and neighboring conductances also play a major role on the impact of a gap junction on a cell's activity. It is well established that gap junctions can both synchronize and desynchronize neuronal activity[77, 86-88], and their role in the emergence and frequency of oscillations is controversial and likely dependent on each neural system[79, 89-92]. Therefore, investigating questions of gap junction physiology can illuminate the workings of a system, although findings should be interpreted with caution when looking into other neural or biological networks. Nevertheless, such investigations can identify what is possible when a neural network utilizes gap junctions, and their impact on its activity.

Over the past decades, neuroscientists have struggled to identify the emergent properties gained from a neural network upon introduction of electrical synapses. Connexin 36 (Cx36) knock-out animal models have been instrumental in this effort, yet did not succeed in providing conclusive answers[93-98]. This is due to the upregulation of other connexins in the knock-out animals leading to decreased, but not absent, electrical connectivity[99, 100].

The work discussed in Paper II includes, to our knowledge, the first systematic comparison of a neural network in the presence of strong Cx36 coupling (up to 0.5 coupling coefficient!), and in the complete absence of gap junctions. The findings of this study revive previous questions of gap junction physiology and its role on network synchronization and oscillation frequency.

1.4   NEURAL ACTIVITY AND NEUROTRANSMITTER RELEASE

Since the identification of the action potential in 1939, it has become apparent that information processing does not follow a linear function between neurons connected through chemical synapses[101-104]. From the discovery of frequency tuning of terminals to respond to impulses arriving at a specific frequency[103, 105], to the identification of the packaging of small neurotransmitters and neuropeptides in small clear and large dense-core vesicles respectively[106-111], the list of rules which governs neural communication is longer than originally thought or often considered in the field of systems neuroscience.

The initial investigations looking into the link between AP firing frequency and dopamine output, focused on the nigrostriatal system due to its prominent role in health and disease[103, 112-115]. In this system, work by Francois Gonon identified the relationship between impulse flow and DA release in the striatum[116]. This seminal work highlighted the importance of gaining information at this level in order to understand a system's output.

Following this reasoning, we pursued the study presented in Paper III which discusses the frequency tuning of neurotransmitter release in TIDA neurons at the level of their terminals in the median eminence (ME).

1.5 THE HYPOTHALAMUS AS A BRAIN CENTER

The hypothalamus is a highly conserved part of the brain across taxa[117-121]. One of the interesting features of this unit, is that it provides the interface of communication between the brain and the pituitary[122, 123] – the "master" endocrine gland responsible for the release of numerous hormones necessary for physiological homeostatic regulation[124-128].

Among the neural groups in control of hormone release from the pituitary, this thesis focuses on the TIDA neurons, which project to the ME where they release DA[129-135]. Ambient levels of DA inhibit the release of prolactin (Prl) from the Prl-releasing cells in the anterior pituitary, the lactotrophs[136-141]. The role of TIDA neurons in the female is well documented[142-152], yet in the male it is obscure[153-156].

In addition, large focus is placed on a subset of ventral premammillary nucleus (PMv) neurons, whose role in social behavior is investigated.

1.6 NEURAL SUBSTRATES OF PARENTAL BEHAVIOR

Hypothalamic neurons are involved in numerous behaviors, such as sleep/wakefulness[157-170], food intake[171-181], parental behavior[182-186] and aggression[187-194]. Innate behaviors in particular have been shown to be orchestrated by neural ensembles present in the hypothalamus.

Parental behavior, defined as the dedication of resources from a parent to the offspring[195], can either be expressed by both parents (biparental strategy)[196-201] or, as occurs in most species, only by one (uniparental strategy)[195, 202]. The evolutionary benefits of a uni- *vs* bi-parental strategy are not evident[203, 204] and, of interest, is the observation that sister species can fall on opposite ends of the spectrum[205].

Several brain areas have been shown to play a role in maternal behavior, including the anteroventral periventricular nucleus[206-208], medial preoptic area (MPOA)[182-184, 202, 209-214], and the amygdala[183, 212, 214-218]. Among these areas the MPOA has attracted a lot of attention, as both the activity of its Prl receptor expressing neurons[209] and the diverse projections of galanin-positive neurons of the MPOA[202] were recently shown to orchestrate most aspects of maternal behavior.

In the present thesis we suggest discrepant TIDA neuron activity found in the male rat and mouse impacts serum Prl levels and tunes activity of the neural circuitry underlying parental behavior. This work offers an insight over a mechanism that can enable or not paternal behavior in a species, suggesting TIDA cells are a neural toggle unit controlling a species' parental strategy.

1.7 NEURAL SUBSTRATES OF AGGRESSION

In a similar way to parental behavior, aggression is another innate behavior which has been shown to be largely controlled via neural groups present in the hypothalamus[219-231], while extra-hypothalamic neurons have been shown to control the valence of aggression[232-248].

Early work using extracellular stimulation defined an area in the hypothalamus that evokes aggression both in cats and rodents, and was therefore named the hypothalamic attack area[194, 249-251]. Menno Kruk's elaborate studies, among others, coupled the site of stimulation with the precise behavior elicited, such as flank attack, tail rattling, biting, social grooming etc[193, 251-258]. Although this work provided a foundation for the neuroscience aggression field, it was prone to criticism due to the methodology utilized to gain this understanding. Electrical stimulation could stimulate fibers of passage and, therefore, it could not be excluded that the neural substrates underlying aggression are extra-hypothalamic.

Elaborate immediate early gene studies provided additional evidence implicating hypothalamic cell groups in aggression[190]. Following this, *in vivo* optogenetics allowed the selective activation of defined neural populations, most often in the rodent brain, and a functional interrogation of those sets of neurons in animal behavior[259-262].

In the present thesis we provide two in-depth investigations on the role of the PMv in intermale and maternal aggression. Additionally, we explore features of aggressive behavior which, while they have been previously discussed, they lack a mechanistic understanding. These include a neural basis of hysteresis in aggression[190, 261, 263, 264], a site in the aggression circuit responsible for conferring a positive valence to aggressive behavior[265-269], and a node which upon activation elicits maternal aggression and is activated by maternal hormones[270-274].

2 AIMS

In its first section (Papers I-III) this thesis aims to deliver answers on neurophysiological principles governing neural function, while in the second section (Papers IV-VI) it aims to address the influence of genetically defined neural clusters in animal behavior.

Specifically, the aims of the first section include the identification of:

- the autoregulation principles used by a group of hypothalamic neurons, which tune their electrophysiological behavior to echoes of their own activity (**Paper I**).

- the role of gap junctions in setting oscillation frequency and a network's electrophysiological activity (**Paper II**).

- the link between firing patterns in the neuroendocrine TIDA neurons and DA release at their terminals (**Paper III**).

The material included in the second section aims to answer:

- how a difference in the electrical coupling of a neural network impacts on the network's output and the expression of a species parental strategy (**Paper IV**).

- the neural network dynamics underlying intermale aggression and hierarchy (**Paper V**).

- how maternal hormones act upon the neural circuit orchestrating aggression and induce maternal aggression in lactating dams (**Paper VI**).

3 METHODS

All methods are described in detail in the individual papers (I-VI). Below is a brief summary of the core methods that were used in this thesis.

3.1 TRANSGENIC ANIMALS AND VIRUSES

While the use of wild-type outbred animals in neurophysiology and behavior provides an obvious benefit since it takes into consideration the genetic variability found in a natural population and therefore serves as a more faithful model for human conditions, transgenic inbred animals have become an indispensable tool in neuroscience research. The development of a powerful genetic toolbox has enabled tissue-specific transgene expression, with timing and location control[275-280], making inbred mouse lines a dominant animal model.

The toolbox largely rests on genetic recombination methods identified in non-mammalian genomes such as the one in bacteriophages[281] and the baker's yeast[282, 283], *Saccharomyces cerevisiae*. The Cre-lox system (identified in bacteriophage P1 which infects *Escherichia coli*) is such an example, and is one of the core tools that enabled the findings discussed in the present thesis. In short, the Cre protein is a recombinase that recognizes a 34 base pair DNA sequence, named as the loxP sites. What enables a large degree of versatility in the Cre-lox system is that based on the location of the loxP sites, the gene of interest can be arranged in a number of ways.

Firstly, if the loxP sites are on the same DNA strand and in opposite orientations, this will result in an inversion of the gene between the loxP sites. This is the primary method that was used in this thesis to enable transgene expression. Breeding a DAT-Cre homozygote mouse line with a floxed-tdTomato mouse line or a floxed-GCaMP3 mouse line, led to the expression of the orange fluorophore (tdTomato) only in cells in which the DA transporter (DAT) promoter was endogenously active. This approach, while it requires no effort from the experimenter into acquiring brain slices with genetically tagged neurons, has the drawback that transient expression of the DAT promoter developmentally will tag neurons permanently, and therefore, as a method, is prone to false positive neural tagging.

Apart from inversion of a gene of interest, the Cre-lox system can also enable deletion or translocation of a gene of interest. If the loxP sites are on the same strand of DNA facing the same direction, the Cre recombinase will excise the piece of DNA between the loxP sites and it will not be maintained. Such an approach is very useful in identifying the influence of a particular gene of interest in genetically tagged neurons, and the gene's role in animal physiology and behavior. Lastly, the Cre-lox system can also enable gene translocation events, a strategy which can be employed by placement of the loxP sites in separate DNA strands.

Of particular importance in this thesis, was the use of the Cre-lox system in combination with viral strategies to induce transgene expression in a subpopulation of genetically tagged neurons with precision both at the temporal and stereotactic coordinate level.

The approach using adeno-associated viruses (AAV) as the carrier of the transgene, flanked by loxP sites to allow its inversion from the anti-sense to the sense configuration in Cre-positive neurons, allowed us to tag the neurons of interest with both fluorophores (eYFP or tdTomato) and/or rhodopsins (ChR2 or eNpHR3). Using the DAT-Cre mouse line and the viral approach mentioned above, we performed functional and anatomical studies of the DAT-Cre positive neurons residing in the arcuate nucleus, shedding light on their function in the control of Prl secretion and their network properties. The same methodology was utilized on a separate set of hypothalamic neurons, a subpopulation of PMv cells which are tyrosine hydroxylase negative but DAT positive which, therefore, can be tagged via the DAT promoter and identified in the DAT-Cre mouse line.

3.2   SLICE PATCH CLAMP ELECTROPHYSIOLOGY

The present thesis contains datasets acquired through patch clamp electrophysiology, offering new insights into neural network function in regard to the TIDA and DAT positive PMv neurons (PMv^{DAT}).

The development of the patch clamp technique by Erwin Neher and Bert Sakmann in the late 1970s-early 1980s[284-287] enabled mechanistic investigations of ion channel physiology and the development of an in-depth understanding of what mediates electrical events and communication between electrogenic cells such as neurons, myocytes and endocrine cells.

Patch clamp electrophysiology was performed in rat or mouse brain slices of 200-400 μm thickness, depending on the experimental question. Both juvenile (p21-28) and adult (2-8 months of age) rodents were used. Current and voltage clamp recordings were acquired using *Axon instruments* (Multiclamp 700B and Digidata 1440A) and were analyzed using custom written Matlab routines.

3.3   FAST-SCAN CYCLIC VOLTAMMETRY (FSCV)

One of the main methods employed in this thesis, allowing us to couple electrophysiological activity with neurotransmitter release at the level of the TIDA terminals, was FSCV. FSCV allows electrochemical measurements at high sampling rate for the acquisition of a voltammogram, which is used to identify the precise nature and concentration of a molecule or neurotransmitter such as DA[288-296]. This makes FSCV a superior method in comparison to other techniques utilized for the same purpose, like amperometry, since it not only allows the recording and quantification of a neurotransmitter's concentration, but also permits its chemical identification[297].

While FSCV has been used both *in vitro* and *in vivo*, in this thesis its application is restricted to brain slice recordings. Additionally, while FSCV can reliably identify most monoamines, in

this thesis we used FSCV for the purpose of recording stimulation-evoked DA release. Cre-dependent ChR2 expression was induced in TIDA neurons following injection of a ChR2-containing AAV one month prior to the recording.

Acquisition of FSCV recordings was performed using a DAGAN CHEM-CLAMP and a custom made digitizer. Tar Heel and HDCV software were used for data acquisition and HDCV and custom made Matlab routines were used for analysis.

3.4 OPTOGENETICS AND BEHAVIOR

Behavioral measurements were central to our investigations, and the advent of optogenetics[298, 299] allowed us to pursue several exciting questions.

In vivo optogenetics experiments were performed following the placement of optic fibers at the appropriate stereotactic coordinates using the Franklin and Paxinos mouse brain atlas[300]. Cre-dependent expression of eYFP, tdTomato, mCherry, ChR2, eNpHR3, diphtheria toxin subunit A, or taCasp3, was induced through stereotactic injection of an AAV vector. All AAVs were purchased from the UPENN and UNC vector cores. Optogenetics-related hardware was acquired from Thorlabs, CNI lasers, and Doric. Custom made digitizers were made using Arduino boards and Labview-based custom written software was developed for photostimulation control.

Behavioral assays including the resident intruder (RI) test, real-time place preference or aversion, sociability test, pup retrieval test (PRT), elevated plus maze, open field test, hierarchy corridor test (HCT), and conditioned place preference were used for addressing relevant questions. The HCT represents a custom adaptation to the widely used test for assessing hierarchy in rodents, the tube test[301, 302], a necessary modification to allow the use of *in vivo* optogenetic manipulation.

4 RESULTS AND DISCUSSION

The first question addressed in this thesis is how a neural network can use its own neurotransmitter to tune its activity. In this work we used the TIDA network as a model system to gain an insight into autoregulation properties that may be relevant to other biological cell networks.

4.1 PAPER I: DOPAMINE AUTORECEPTOR REGULATION OF A HYPOTHALA-MIC DOPAMINERGIC NETWORK

Rat TIDA neurons exhibit a distinct electrophysiological activity, with the expression of robust, high-amplitude oscillations at 0.05 Hz at room temperature[56] and ca 0.17 Hz in near-physiological ($34^{o}C$) temperature. We first examined whether TIDA neurons respond to their own neurotransmitter, DA. Application of DA – or D2R agonists - decreased the oscillation frequency, having a distinct impact on phases 1 and 3 of the oscillation (Fig. 1).
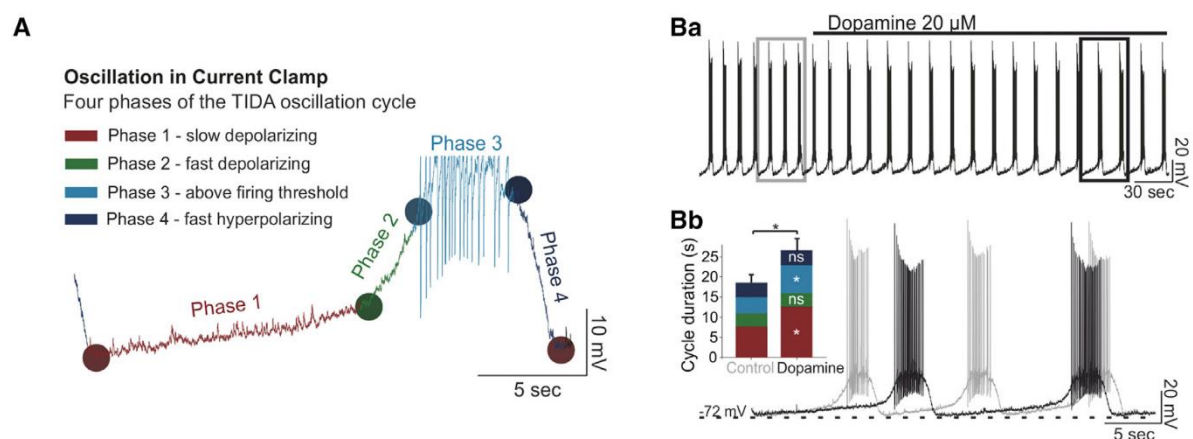

Figure 1. Dopamine decreases oscillation frequency of the TIDA neuron oscillation. A. The TIDA neuron oscillation frequency can be broken down in four phases. B. TIDA neuron oscillation frequency during baseline and following application of DA.

Interestingly, application of D2-type DA receptor blockers led to abolishment of the oscillation, while DAT blockade recapitulated the exogenous DA bath-application effect. DA was found to have both pre- and post-synaptic effects, with part of the latter mediated via a decrease in high-voltage Ca^{2+} currents (Fig. 2).
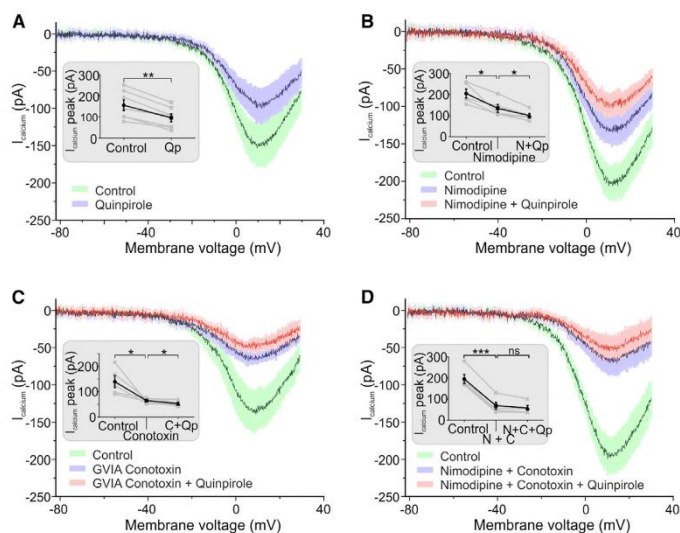
In summary, this study describes the neurophysiological mechanisms utilized by the TIDA network to tune its own activity using perisomatic release of DA.

Figure 2. D2-type dopamine receptor activation decreases Ca^{2+} currents through N- and L-type Ca^{2+} currents. A. DA decreases Ca^{2+} currents in TIDA neurons. B. DA decreases Ca^{2+} currents in the presence of an L-type Ca^{2+} channel blocker. C. DA decreases Ca^{2+} currents in the presence of an N-type Ca^{2+} channel blocker. D. DA does not alter Ca^{2+} currents in the presence of L- and N-type Ca^{2+} channel blockers.

10

The second question addressed in this thesis deals with the characterization of a neural network in the presence *vs* absence of electrical coupling. This is used to gain an understanding of the emergent properties acquired by a network when electrically coupled.

4.2   PAPER II: NETWORK OSCILLATION RULES IMPOSED BY SPECIES-SPECIFIC ELECTRICAL COUPLING

Here we came across an unexpected finding, with the discovery that TIDA neuron oscillations are fast in the male mouse and slow in the male rat (Fig. 3).
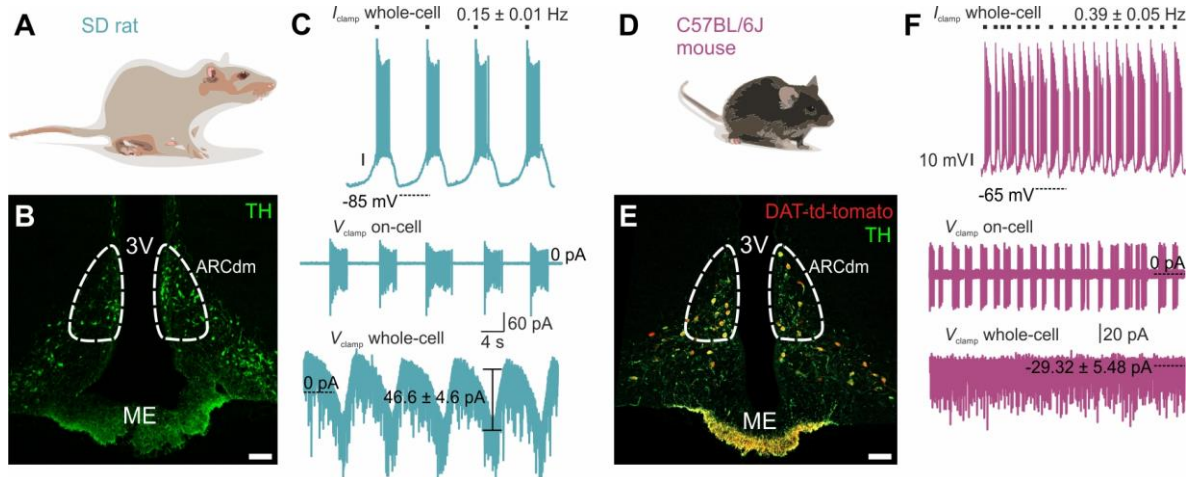
Figure 3. TIDA neuron activity in the male rat and mouse. A-C. Rat TIDA neuron electrophysiology in the rat ARCdm. D-F. Mouse TIDA neuron electrophysiology in the mouse ARCdm.

Following an in-depth electrophysiological interrogation of the membrane properties and electrical connectivity, we identified strong gap junction coupling in rat *vs* the complete absence of gap junctions in mouse TIDA neurons. Lastly, we identified that gap junctions give rise to a slower oscillation frequency assumed collectively by the TIDA neuron population while, in an uncoupled state, these cells oscillate at a faster frequency (Fig. 4).
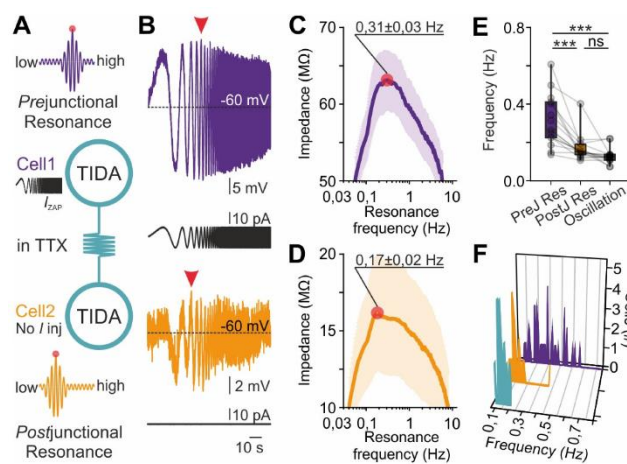
Figure 4. Gap junction resonance dictates network oscillation frequency. A. Schematic of experimental design. B. Raw data measurements. C-F. Pre- and post-junctional resonance frequency, raw data (C and D) and quantification (E and F).

This study is the first to our knowledge to experimentally address the impact of electrical connectivity in a neural network in the presence of strong electrical coupling and in its complete absence. As such, it provides a new answer to a long-standing debate over the role of gap junctions in neural oscillations and their relevance in the activity of biological systems[88, 94, 303-307].

This study also raised the question of how the distinct electrophysiological activity impacts release at the TIDA neuron terminals. The study discussed in 4.3 was designed to address this point.

Following the identification of different electrophysiological patterns in male rat *vs* mouse TIDA neurons, we pursued an examination of the impact of distinct firing patterns and frequencies at the level of DA release at the TIDA terminals in the ME.

4.3 PAPER III: DOPAMINE RELEASE DYNAMICS IN THE TUBEROINFUNDIBULAR DOPAMINE SYSTEM

Firstly, we aimed to identify the baseline levels of DA released in the ME, since both rat and mouse TIDA neurons are spontaneously active. However, recordings of DA release in the ME in baseline conditions yielded no evidence for spontaneous DA release in this preparation, likely due to a severed axonal path between ARCdm and ME. However, following transduction of TIDA neurons with ChR2, optically evoked DA release was found to be stable over time (Fig. 5), allowing the use of this model to address the relationship between firing frequency and DA release in TIDA neurons.
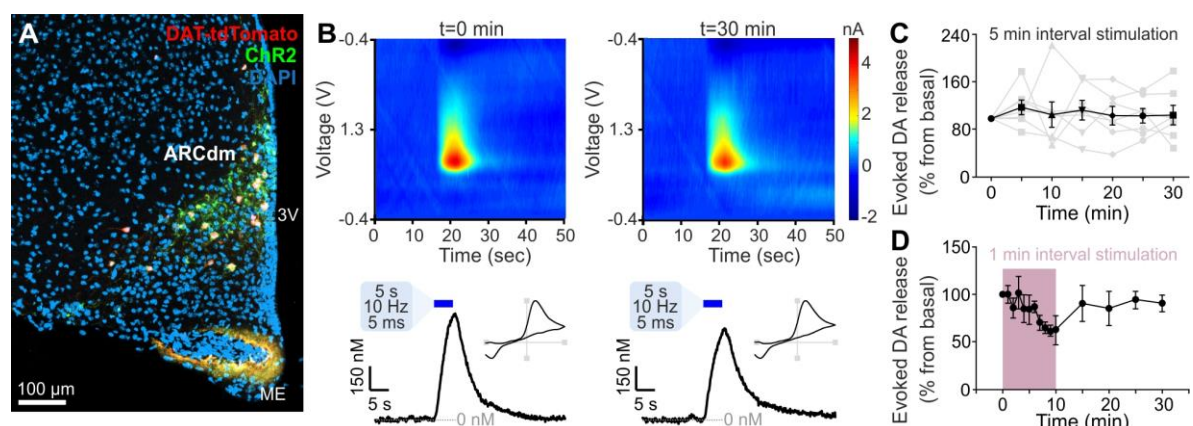

Figure 5. Optogenetically evoked DA release in the ME is stable over time. A. ChR2 expression in TIDA neurons in a DAT-tdTomato mouse line, and in their terminals in the ME. B. FSCV recordings at t=0 min and at t=30 min. C, D. Quantification of evoked DA release following different photostimulation intervals.

Following the identification of the TIDA ChR2 expressing terminals in the ME as a reliable model to study DA release dynamics, we used a photostimulation paradigm to investigate the impact of firing frequency on neurotransmitter release. Here we found that in the TIDA system, optimal release following a brief (3 s) bout of activity, occurs at 10 Hz (Fig. 6). This contrasts other systems such as, for example, the nigrostriatal projections with optimal DA release dynamics at 40 Hz[308, 309].
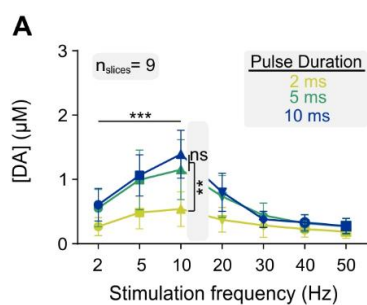

Figure 6. Optimal DA release from ME terminals occurs at 10 Hz. A. Quantification of DA release *vs* photostimulation frequency and pulse width.

In addition, we found that endogenous firing of mouse TIDA neurons *in vitro* occurs at 10 Hz, and firing frequencies higher than 20 Hz were never recorded (n=100 TIDA neurons). Furthermore, we provide evidence of a functional DAT at the level of the TIDA terminals in the ME, and identified that TIDA neurons can release DA at the perisomatic level in the ARCdm.

This study provides a mechanistic interrogation of the DA release dynamics in the TIDA system, and enables an understanding of how distinct firing patterns can impact the system's output.

In the last study focused on understanding the TIDA system in this thesis, we provide a link between the distinct TIDA electrophysiological activity patterns in the male rat and mouse, and paternal behavior in each species.

4.4 PAPER IV: NEUROHORMONAL BASIS OF PATERNAL BEHAVIOR

Following the identification of different activity patterns in the TIDA system in the male rat and mouse (Paper II), we now examined the link between *oscillation* frequency and DA release. Using FSCV, we found that the rat frequency (0.2 Hz) can sustain DA release over time, in striking contrast with the mouse oscillation frequency (0.4 Hz) which leads only to transient DA release (Fig. 7).
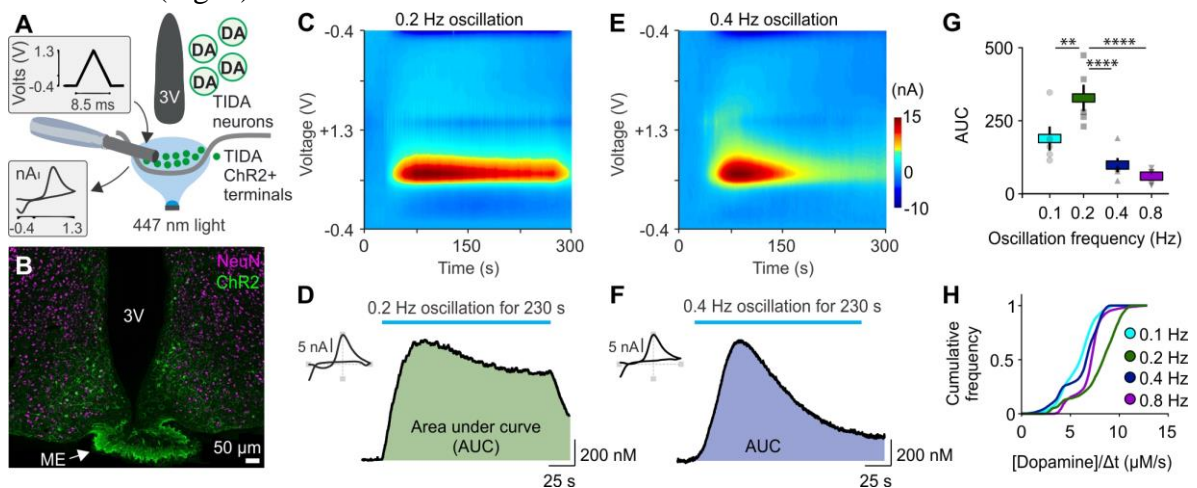

Figure 7. A slow 0.2 Hz TIDA oscillation can sustain DA release over time, in contrast to faster oscillation frequencies. A. Schematic of experimental design. B. ChR2 expression at the TIDA terminals. C-F. Investigation of DA release in comparison to oscillation frequency.

This finding inspired a follow-up experiment in which we identified that the *in vivo* serum Prl concentrations differ in the male rat and mouse, as predicted by the finding in Fig. 7. Additionally, the low *vs* high Prl levels found in the male rat and mouse respectively correlated to low *vs* high Prl receptor activation in the MPOA. MPOA has been implicated in parental behavior[202, 208, 209, 212, 310] and, following this link, we identified that rats and mice fall at opposite ends of the parental behavior spectrum (Fig. 8).
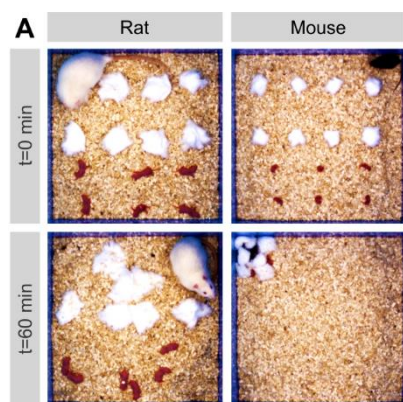

Figure 8. Paternal behavior as exhibited by rat and mouse sires. A. Example snapshots of the pup retrieval test at the beginning (0 min) and end of the test (60 min). Note that only mouse sires have performed pup retrieval and nesting.

To establish a causal link, mouse TIDA neuron activity was controlled using optogenetics, where application of the 0.2 Hz (rat) oscillation frequency resulted in a decrease of Prl levels in mouse sires and impairment of the expression of paternal behavior. Meanwhile i.p. injection of Prl induced aspects of paternal behavior in rat sires, similar to previous observations[311].

In summary, this work identifies neural and endocrine mechanisms that determine a rodent species' parental strategy. It remains to be tested if the same mechanism is utilized universally to determine parental strategy in other mammalian species.

The following study presents a switch of gear in the present thesis, as it involves the investigation of a group of hypothalamic neurons in control of aggressive behavior – the PMv^{DAT} cells.

4.5 PAPER V: A NEURAL NETWORK FOR INTERMALE AGGRESSION TO ESTABLISH SOCIAL HIERARCHY

An observation that attracted our interest was the expression of DAT in a subset of PMv tyrosine hydroxylase-negative cells (Fig. 1A-C). Following previous indications of the role of the PMv in aggression[190, 312], we pursued *in vitro* and *in vivo* experiments to test the role of PMv^{DAT} cells in mouse social behavior. The use of a RI conditioning paradigm together with the RI test, led to our first observation, with immediate early gene studies indicating activation of PMv^{DAT} cells following episodes of aggression (Fig. 9D, E).
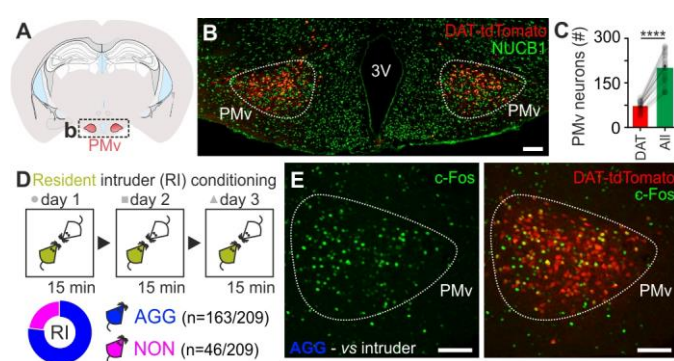

Figure 9. PMv^{DAT} neurons are activated during aggression. A-C. A subset of PMv cells express DAT. D. RI conditioning paradigm employed in this study. E. c-Fos immunoreactivity in PMv following an aggression episode.

Photoactivation of PMv^{DAT} neurons initiated attack, whereas photoinhibition stopped ongoing aggressive episodes. Importantly, PMv^{DAT} cells were found to have membrane properties and network connectivity which permits feedforward excitation.

Distinct glutamatergic projections of PMv^{DAT} neurons to the ventromedial hypothalamus and supramammillary nucleus were found to drive an aggressive and rewarding component respectively, implicating the PMv as a neural structure which can simultaneously drive multiple aggression-related behaviors.
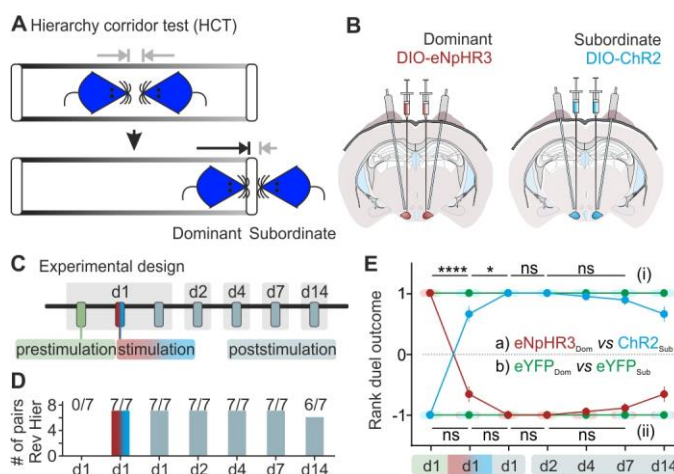
Figure 10. PMv^{DAT} neuron activity manipulation leads to a long-lasting switch in intermale hierarchy. A-C. Experimental design of the HCT. D. Collective results following a switch in the hierarchical pattern. E. Quantification of social rank in the HCT test during the test period of two weeks.

Lastly, to test the role of PMv^{DAT} neurons in a functional aggression context, we showed that manipulation of these neurons' activity in males competing for social status led to an irreversible switch of the hierarchy between them, an effect lasting up to the maximal tested period of two weeks (Fig. 10A-E).

These data identify a prominent role of PMv^{DAT} neurons in aggression, and add an important node in the neural circuit in control of aggressive behavior.

The last study included in this thesis was inspired by the identification of PMv[DAT] cells in intermale aggression, which opened up the possibility of a role of PMv[DAT] neurons in maternal aggression. The influence of maternal hormones on these cells was also examined in depth.

4.6 PAPER VI: CONTROL OF MATERNAL AGGRESSION VIA MATERNAL-HORMONE SENSITIVE HYPOTHALAMIC NEURONS

Similarly to what was found with immediate early gene studies following an intermale aggression episode in PMv[DAT] neurons, here we identified that PMv[DAT] cells in the dam are also activated following the expression of maternal aggression (Fig. 11).
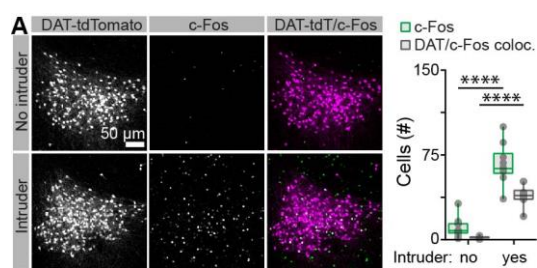

Figure 11. PMv[DAT] neurons are activated in maternal aggression. A. c-Fos immunoreactivity in PMv and PMv[DAT] cells following the RI test, using lactating dams as residents.

Transfecting PMv[DAT] neurons with ChR2, enabled us to initiate aggressive episodes in lactating dams against both male and female intruders, whereas photoinhibition via eNpHR3 stopped ongoing episodes.

Additionally, a genetically mediated cell ablation using Cre-dependent taCasp3 expression in PMv[DAT] cells led to a decrease in the expression of maternal aggression (Fig. 12).
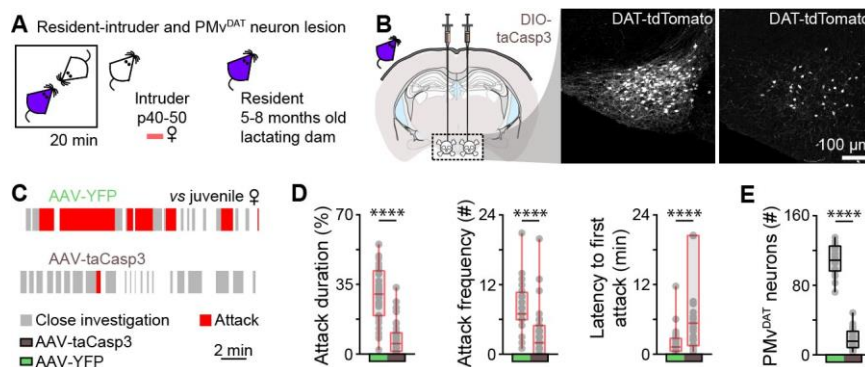

Figure 12. PMv[DAT] neuron lesion leads to a decrease in the expression of maternal aggression. A. Schematic of the experimental design. B. Representative PMv confocal images injected with control and DIO-taCasp3 virus. C. Behavioral raster plots in control and PMv-lesioned conditions. D. Quantification of aggression parameters. E. Quantification of the extent of the PMv[DAT] neuron lesion.

Importantly, using slice electrophysiology, PMv[DAT] neurons were found to be responsive to maternal hormone bath application. Both Prl and oxytocin were shown to depolarize PMv[DAT] neurons, likely through similar conductances.

To test the involvement of these neurons in other maternal behaviors, such as maternal care, we used the PRT and photoactivation with ChR2. Surprisingly, photostimulation during the PRT impaired maternal care, suggestive of the role of these cells towards promoting aggressive behavior rather than a collective maternal repertoire.

This study provides insights into the neural correlates of maternal aggression, and identifies the PMv[DAT] cells as a site of maternal hormone action to induce aggressive behavior in the female.

5 CONCLUSION AND FUTURE PERSPECTIVES

The initial part of this thesis (Papers I-III) aimed at increasing our understanding of neural function at the cellular, ion channel and neurotransmitter release level, and this effort amounted to the identification of novel principles of autoregulation, gap junction physiology and DA release properties in the TIDA system. Such an approach is essential in the continuous effort of the neuroscience community to gain insights into neural function, and highlights that a neuron's output is subject to multiple concurrent neurobiological rules and not merely a linear reflection of AP frequency.

The second part of the thesis (Papers IV-VI) then attempts to establish a link between TIDA neurons and parental behavior (Paper IV), while Papers V and VI identify a prominent role of PMv[DAT] neurons in the circuit underlying multiple forms of aggression.

The work discussed in Paper IV discusses a mechanistic interrogation of the lactotropic axis and its role in paternal behavior. Given that most mammalian species are maternally uniparental[195], and sister species can be found to follow opposite parental strategies[205], this work provides an understanding of how TIDA cell activity can tilt the parental circuit in a species towards high or low activation, ultimately having an impact on a rodent's parental behavior. It would be of great interest to identify the TIDA network activity in other uni- and biparental mammals, and to investigate whether gap junctions are the core variable influencing TIDA neuron activity and parental strategy in species other than rodents. Paper IV provides a conceptual advance in which, if our findings can be extrapolated, evolution can tilt a species parental strategy to increase its fitness based on environmental needs simply through the control of a single gene in TIDA neurons – that of Cx36. Rodents previously used in behavioral neuroscience such as the California mouse, the prairie and mountain vole would be excellent models to pursue these questions[313-318].

The experiments presented in Paper V implicate PMv[DAT] cells in intermale aggression and hierarchy. Importantly, they identify the PMv as an important node in the aggression circuit and highlight its interconnectivity with neural nodes previously associated with aggression or reward. A unique part of this work is the identification of PMv[DAT] cells in a functional aggression context, using an intermale competition test for social rank (the HCT). In addition to demonstrating the initiation or termination of aggression episodes using *in vivo* photostimulation, we provide insights into the temporal aspects that follow the establishment and re-establishment of intermale hierarchy. We show that, following the reversal of hierarchical status between two competing males, hierarchy remains inversed for the maximal tested period of two weeks. In nature following the establishment of hierarchy between two males, the individuals often do not challenge each other for large periods of time, usually defined by the initiation of the next breeding season[319-324]. An exciting future experiment, with the use of the HCT paradigm as performed in Paper V, is to identify the neural mechanisms that enable "storage" of the information with regard to an individual's social rank, and those that underlie the temporal aspects that accompany the decision making underlying challenging and attempting the gain of hierarchical status.

The project recounted in Paper VI identifies the influence of maternal hormones (Prl and oxytocin) in activating PMv^{DAT} cells. Additionally, we show that optogenetic photoactivation or photoinhibition of these neurons can induce or stop maternal aggression, while it impairs other maternal behaviors. These findings pinpoint how maternal hormones can activate a "dormant" neural circuit, allowing the conditional expression of aggressive behavior in female mice. Such a mechanism is of great interest since it involves neural plasticity in adulthood in a neural circuit that permits the expression of an innate behavior with minimal influence of learning from conspecifics.

In summary, this thesis attempts to introduce and develop concepts on what drives physiological neural network activity and meaningful behavioral outcome. In our view, this is a necessary step prior to an attempt towards tackling pathophysiology in the human brain, a goal that for every generation of neuroscientists is getting closer to becoming tangible.

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7 REFERENCES

1. Greene, J.C. Darwinism and moral purpose: darwin and the emergence of evolutionary theories of mind and behavior. *Science* **239**, 198-199 (1988).

2. Ghiselin, M.T. Darwin and Evolutionary Psychology: Darwin initiated a radically new way of studying behavior. *Science* **179**, 964-968 (1973).

3. Darwin, C. On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. *The British and foreign medico-chirurgical review* **25**, 367-404 (1860).

4. Sherrington, C.S. Note toward the Localisation of the Knee-Jerk. *Br Med J* **1**, 545 (1892).

5. Sherrington, C.S. Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *The Journal of physiology* **40**, 28-121 (1910).

6. Brown, T.G. & Sherrington, C.S. The rule of reflex response in the limb reflexes of the mammal and its exceptions. *The Journal of physiology* **44**, 125-130 (1912).

7. Sherrington, C.S. Further observations on the production of reflex stepping by combination of reflex excitation with reflex inhibition. *The Journal of physiology* **47**, 196-214 (1913).

8. Langley, J.N. & Sherrington, C.S. Secondary Degeneration of Nerve Tracts following removal of the Cortex of the Cerebrum in the Dog. *The Journal of physiology* **5**, 49-126 125 (1884).

9. Sherrington, C.S. On Secondary and Tertiary Degenerations in the Spinal Cord of the Dog. *The Journal of physiology* **6**, 177-292 110 (1885).

10. Sherrington, C.S. On Nerve-Tracts degenerating secondarily to Lesions of the Cortex Cerebri. *The Journal of physiology* **10**, 429-432 (1889).

11. Sherrington, C.S. & Ballance, C.A. On Formation of Scar-tissue. *The Journal of physiology* **10**, 550-576 558 (1889).

12. Shepherd, G.M. The neuron doctrine: a revision of functional concepts. *The Yale journal of biology and medicine* **45**, 584-599 (1972).

13. Gold, I. & Stoljar, D. A neuron doctrine in the philosophy of neuroscience. *Behav Brain Sci* **22**, 809-830; discussion 831-869 (1999).

14. Yuste, R. From the neuron doctrine to neural networks. *Nature reviews. Neuroscience* **16**, 487-497 (2015).

15. Cole, K.S. & Curtis, H.J. Electric Impedance of the Squid Giant Axon during Activity. *The Journal of general physiology* **22**, 649-670 (1939).

16. Eccles, J.C. Facilitation and inhibition in the superior cervical ganglion. *The Journal of physiology* **85**, 207-238 203 (1935).

17. Eccles, J.C. The action potential of the superior cervical ganglion. *The Journal of physiology* **85**, 179-206 172 (1935).

18. Eccles, J.C. Synaptic potentials and transmission in sympathetic ganglion. *The Journal of physiology* **101**, 465-483 (1943).

19. Eccles, J.C. Synaptic potentials of motoneurones. *Journal of neurophysiology* **9**, 87-120 (1946).

20. Hodgkin, A.L. The relation between conduction velocity and the electrical resistance outside a nerve fibre. *The Journal of physiology* **94**, 560-570 (1939).

21. Hodgkin, A.L., Huxley, A.F. & Katz, B. Measurement of Current-Voltage Relations in the Membrane of the Giant Axon of Loligo. *J Physiol-London* **116**, 424-448 (1952).

22. Hodgkin, A.L. & Huxley, A.F. Propagation of Electrical Signals Along Giant Nerve Fibres. *Proc R Soc Ser B-Bio* **140**, 177-183 (1952).

23. Hodgkin, A.L. & Huxley, A.F. Movement of Sodium and Potassium Ions during Nervous Activity. *Cold Spring Harbor symposia on quantitative biology* **17**, 43-52 (1952).

24. Hodgkin, A.L. & Huxley, A.F. The Components of Membrane Conductance in the Giant Axon of Loligo. *J Physiol-London* **116**, 473-496 (1952).

25. Hodgkin, A.L., Huxley, A.F. & Katz, B. Ionic Currents Underlying Activity in the Giant Axon of the Squid. *Archives des sciences physiologiques* **3**, 129-150 (1949).

26. Hodgkin, A.L. & Huxley, A.F. Potassium Leakage from an Active Nerve Fibre. *J Physiol-London* **106**, 341-& (1947).

27. Hodgkin, A.L. & Huxley, A.F. Resting and Action Potentials in Single Nerve Fibres. *J Physiol-London* **104**, 176-195 (1945).

28. Hodgkin, A.L. & Katz, B. The effect of sodium ions on the electrical activity of giant axon of the squid. *The Journal of physiology* **108**, 37-77 (1949).

29. Fatt, P. & Katz, B. Spontaneous Subthreshold Activity at Motor Nerve Endings. *J Physiol-London* **117**, 109-128 (1952).

30. Rall, W. Membrane potential transients and membrane time constant of motoneurons. *Experimental neurology* **2**, 503-532 (1960).

31. Rall, W. Branching dendritic trees and motoneuron membrane resistivity. *Experimental neurology* **1**, 491-527 (1959).

32. Rall, W. Membrane time constant of motoneurons. *Science* **126**, 454 (1957).

33. Piccinini, G. The first computational theory of mind and brain: A close look at McCulloch and Pitts's "logical calculus of ideas immanent in nervous activity". *Synthese* **141**, 175-215 (2004).

34. Mcculloch, W.S. & Pitts, W. A Logical Calculus of the Ideas Immanent in Nervous Activity (Reprinted from Bulletin of Mathematical Biophysics, Vol 5, Pg 115-133, 1943). *Bulletin of mathematical biology* **52**, 99-115 (1990).

35. Lim, J.H. & Choi, K.W. Induction and autoregulation of the anti-proneural gene Bar during retinal neurogenesis in Drosophila. *Development* **131**, 5573-5580 (2004).

36. Li, S.Y. & Pelletier, G. Involvement of an Autoregulatory Mechanism for the Regulation of Gonadotropin-Releasing-Hormone (Gnrh) Gene-Expression in Neurons in the Rat Preoptic Area. *Neuroscience letters* **174**, 61-63 (1994).

37. Laoye, B.J. et al. Dopamine binds calmodulin during autoregulation of dopaminergic D2 receptor signaling through CaMKII alpha-calmodulin complex. *J Recept Sig Transd* **36**, 271-277 (2016).

38. Pineyro, G. & Blier, P. Autoregulation of serotonin neurons: Role in antidepressant drug action. *Pharmacological reviews* **51**, 533-591 (1999).

39. Balthazart, J., Foidart, A., Surlemont, C., Harada, N. & Naftolin, F. Neuroanatomical Specificity in the Autoregulation of Aromatase-Immunoreactive Neurons by Androgens and Estrogens - an Immunocytochemical Study. *Brain research* **574**, 280-290 (1992).

40. Bacci, A. & Huguenard, J.R. Enhancement of spike-timing precision by autaptic transmission in neocortical inhibitory interneurons. *Neuron* **49**, 119-130 (2006).

41. Tamas, G., Buhl, E.H. & Somogyi, P. Massive autaptic self-innervation of GABAergic neurons in cat visual cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **17**, 6352-6364 (1997).

42. Bekkers, J.M. Synaptic transmission: functional autapses in the cortex. *Current biology : CB* **13**, R433-435 (2003).

43. Leviel, V., Cheramy, A. & Glowinski, J. Role of the dendritic release of dopamine in the reciprocal control of the two nigro-striatal dopaminergic pathways. *Nature* **280**, 236-239 (1979).

44. Rice, M.E. & Patel, J.C. Somatodendritic dopamine release: recent mechanistic insights. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **370** (2015).

45. Herman, J.P., McKlveen, J.M., Solomon, M.B., Carvalho-Netto, E. & Myers, B. Neural regulation of the stress response: glucocorticoid feedback mechanisms. *Brazilian Journal of Medical and Biological Research* **45**, 292-298 (2012).

46. Gonzalez-Hoyuela, M., Barbas, J.A. & Rodriguez-Tebar, A. The autoregulation of retinal ganglion cell number. *Development* **128**, 117-124 (2001).

47. Fu, W.M. & Liu, J.J. Regulation of acetylcholine release by presynaptic nicotinic receptors at developing neuromuscular synapses. *Molecular pharmacology* **51**, 390-398 (1997).

48. Williams, P.D.E. et al. Serotonin Disinhibits a Caenorhabditis elegans Sensory Neuron by Suppressing Ca(2+)-Dependent Negative Feedback. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **38**, 2069-2080 (2018).

49. Yeo, S.H., Clarkson, J. & Herbison, A.E. Kisspeptin-gpr54 signaling at the GnRH neuron is necessary for negative feedback regulation of luteinizing hormone secretion in female mice. *Neuroendocrinology* **100**, 191-197 (2014).

50. Christian, C.A., Glidewell-Kenney, C., Jameson, J.L. & Moenter, S.M. Classical estrogen receptor alpha signaling mediates negative and positive feedback on gonadotropin-releasing hormone neuron firing. *Endocrinology* **149**, 5328-5334 (2008).

51. Serizawa, S. et al. Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. *Science* **302**, 2088-2094 (2003).

52. Shi, W.X., Smith, P.L., Pun, C.L., Millet, B. & Bunney, B.S. D1-D2 interaction in feedback control of midbrain dopamine neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **17**, 7988-7994 (1997).

53. Miyazaki, T. & Lacey, M.G. Presynaptic inhibition by dopamine of a discrete component of GABA release in rat substantia nigra pars reticulata. *The Journal of physiology* **513 (Pt 3)**, 805-817 (1998).

54.   Koga, E. & Momiyama, T. Presynaptic dopamine D2-like receptors inhibit excitatory transmission onto rat ventral tegmental dopaminergic neurones. *The Journal of physiology* **523 Pt 1**, 163-173 (2000).

55.   Federici, M., Natoli, S., Bernardi, G. & Mercuri, N.B. Dopamine selectively reduces GABA(B) transmission onto dopaminergic neurones by an unconventional presynaptic action. *The Journal of physiology* **540**, 119-128 (2002).

56.   Lyons, D.J., Horjales-Araujo, E. & Broberger, C. Synchronized network oscillations in rat tuberoinfundibular dopamine neurons: switch to tonic discharge by thyrotropin-releasing hormone. *Neuron* **65**, 217-229 (2010).

57.   Pereda, A.E. Electrical synapses and their functional interactions with chemical synapses. *Nature Reviews Neuroscience* **15**, 250-263 (2014).

58.   Wolburg, H. & Kurz-Isler, G. Dynamics of gap junctions between horizontal cells in the goldfish retina. *Experimental brain research* **60**, 397-401 (1985).

59.   Quick, D.C. & Johnson, R.G. Gap junctions and rhombic particle arrays in planaria. *Journal of ultrastructure research* **60**, 348-361 (1977).

60.   Flower, N.E. Invertebrate gap junctions. *Journal of cell science* **25**, 163-171 (1977).

61.   Nadol, J.B., Jr., Mulroy, M.J., Goodenough, D.A. & Weiss, T.F. Tight and gap junctions in a vertebrate inner ear. *The American journal of anatomy* **147**, 281-301 (1976).

62.   Sipe, J.C. & Moore, R.Y. Astrocytic gap junctions in the rat lateral hypothalamic area. *The Anatomical record* **185**, 247-251 (1976).

63.   Coggeshall, R.E. Gap junctions between identified glial cells in the leech. *Journal of neurobiology* **5**, 463-467 (1974).

64.   Cobb, J.L. Gap junctions in the heart of teleost fish. *Cell and tissue research* **154**, 131-134 (1974).

65.   Sloper, J.J. Gap junctions between dendrites in the primate neocortex. *Brain research* **44**, 641-646 (1972).

66.   Hand, A.R. & Gobel, S. The structural organization of the septate and gap junctions of Hydra. *The Journal of cell biology* **52**, 397-408 (1972).

67.   Silverblatt, F.J. & Bulger, R.E. Gap junctions occur in vertebrate renal proximal tubule cells. *The Journal of cell biology* **47**, 513-515 (1970).

68.   Zandt, B.J., Veruki, M.L. & Hartveit, E. Electrotonic signal processing in AII amacrine cells: compartmental models and passive membrane properties for a gap junction-coupled retinal neuron. *Brain structure & function* **223**, 3383-3410 (2018).

69.   Rackauskas, M., Neverauskas, V. & Skeberdis, V.A. Diversity and properties of connexin gap junction channels. *Medicina (Kaunas)* **46**, 1-12 (2010).

70.   Srinivas, M. et al. Functional properties of channels formed by the neuronal gap junction protein connexin36. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **19**, 9848-9855 (1999).

71.   Bukauskas, F.F., Elfgang, C., Willecke, K. & Weingart, R. Biophysical properties of gap junction channels formed by mouse connexin40 in induced pairs of transfected human HeLa cells. *Biophysical journal* **68**, 2289-2298 (1995).

72.   Bukauskas, F.F. & Weingart, R. Temperature dependence of gap junction properties in neonatal rat heart cells. *Pflugers Archiv : European journal of physiology* **423**, 133-139 (1993).

73.   Chanson, M. & Spray, D.C. Gating and Single Channel Properties of Gap Junction Channels in Hepatopancreatic Cells of Procambarus clarkii. *The Biological bulletin* **183**, 341-342 (1992).

74.   Jabr, R.I. et al. Regulation of gap junction conductance by calcineurin through Cx43 phosphorylation: implications for action potential conduction. *Pflugers Archiv : European journal of physiology* **468**, 1945-1955 (2016).

75.   Magyar, J. et al. Role of gap junction channel in the development of beat-to-beat action potential repolarization variability and arrhythmias. *Current pharmaceutical design* **21**, 1042-1052 (2015).

76.   Shinozaki, T., Naruse, Y. & Cateau, H. Gap junctions facilitate propagation of synchronous firing in the cortical neural population: a numerical simulation study. *Neural networks : the official journal of the International Neural Network Society* **46**, 91-98 (2013).

77.   Zahid, T. & Skinner, F.K. Predicting synchronous and asynchronous network groupings of hippocampal interneurons coupled with dendritic gap junctions. *Brain research* **1262**, 115-129 (2009).

78.   Di Garbo, A., Panarese, A. & Chillemi, S. Gap junctions promote synchronous activities in a network of inhibitory interneurons. *Biosystems* **79**, 91-99 (2005).

79.   Lewis, T.J. & Rinzel, J. Self-organized synchronous oscillations in a network of excitable cells coupled by gap junctions. *Network* **11**, 299-320 (2000).

80.   Delmar, M. Gap junctions as active signaling molecules for synchronous cardiac function. *Journal of cardiovascular electrophysiology* **11**, 118-120 (2000).

81.   Desplantez, T., Halliday, D., Dupont, E., Severs, N.J. & Weingart, R. Influence of v5/6-His tag on the properties of gap junction channels composed of connexin43, connexin40 or connexin45. *The Journal of membrane biology* **240**, 139-150 (2011).

82.   Lin, X. et al. Connexin40 and connexin43 determine gating properties of atrial gap junction channels. *Journal of molecular and cellular cardiology* **48**, 238-245 (2010).

83.   Valiunas, V. Biophysical properties of connexin-45 gap junction hemichannels studied in vertebrate cells. *The Journal of general physiology* **119**, 147-164 (2002).

84.   Manthey, D. et al. Intracellular domains of mouse connexin26 and -30 affect diffusional and electrical properties of gap junction channels. *The Journal of membrane biology* **181**, 137-148 (2001).

85.   Hopperstad, M.G., Srinivas, M. & Spray, D.C. Properties of gap junction channels formed by Cx46 alone and in combination with Cx50. *Biophysical journal* **79**, 1954-1966 (2000).

86.   Sinfield, J.L. & Collins, D.R. Induction of synchronous oscillatory activity in the rat lateral amygdala in vitro is dependent on gap junction activity. *The European journal of neuroscience* **24**, 3091-3095 (2006).

87.   Ikegami, K. et al. Evidence of involvement of neurone-glia/neurone-neurone communications via gap junctions in synchronised activity of KNDy neurones. *Journal of neuroendocrinology* **29** (2017).

88.   Leznik, E. & Llinas, R. Role of gap junctions in synchronized neuronal oscillations in the inferior olive. *Journal of neurophysiology* **94**, 2447-2456 (2005).

89.   Orlowska-Feuer, P., Jeczmien-Lazur, J.S., Szkudlarek, H.J. & Lewandowski, M.H. Retinal gap junctions are involved in rhythmogenesis of neuronal activity at remote locations - Study on infra-slow oscillations in the rat olivary pretectal nucleus. *Neuroscience* **339**, 150-161 (2016).

90.   Simoes de Souza, F.M. & De Schutter, E. Robustness effect of gap junctions between Golgi cells on cerebellar cortex oscillations. *Neural systems & circuits* **1**, 7 (2011).

91.   Maex, R. & De Schutter, E. Mechanism of spontaneous and self-sustained oscillations in networks connected through axo-axonal gap junctions. *The European journal of neuroscience* **25**, 3347-3358 (2007).

92.   Traub, R.D. et al. Gap junctions between interneuron dendrites can enhance synchrony of gamma oscillations in distributed networks. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **21**, 9478-9486 (2001).

93.   Chojnacka, K. et al. Expression of the androgen receptor in the testis of mice with a Sertoli cell specific knock-out of the connexin 43 gene (SCCx43KO(-/-)). *Reproductive biology* **12**, 341-346 (2012).

94.   Buhl, D.L., Harris, K.D., Hormuzdi, S.G., Monyer, H. & Buzsaki, G. Selective impairment of hippocampal gamma oscillations in connexin-36 knock-out mouse in vivo. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **23**, 1013-1018 (2003).

95.   Munger, S.J. et al. Segregated Foxc2, NFATc1 and Connexin expression at normal developing venous valves, and Connexin-specific differences in the valve phenotypes of Cx37, Cx43, and Cx47 knockout mice. *Dev Biol* **412**, 173-190 (2016).

96.   Lin, L. et al. Ultrastructural pathological changes in the cochlear cells of connexin 26 conditional knockout mice. *Mol Med Rep* **8**, 1029-1036 (2013).

97.   Giese, S. et al. Sertoli-cell-specific knockout of connexin 43 leads to multiple alterations in testicular gene expression in prepubertal mice. *Disease models & mechanisms* **5**, 895-913 (2012).

98.   Ramos, A.T. et al. Remyelination in experimentally demyelinated connexin 32 knockout mice. *Arquivos de neuro-psiquiatria* **67**, 488-493 (2009).

99.   De Zeeuw, C.I. et al. Deformation of network connectivity in the inferior olive of connexin 36-deficient mice is compensated by morphological and electrophysiological changes at the single neuron level. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **23**, 4700-4711 (2003).

100.   Anzini, P. et al. Structural abnormalities and deficient maintenance of peripheral nerve myelin in mice lacking the gap junction protein connexin 32. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **17**, 4545-4551 (1997).

101.   Bethge, M., Rotermund, D. & Pawelzik, K. Optimal neural rate coding leads to bimodal firing rate distributions. *Network* **14**, 303-319 (2003).

102.   Gordon, T., Tyreman, N., Rafuse, V.F. & Munson, J.B. Limited plasticity of adult motor units conserves recruitment order and rate coding. *Progress in brain research* **123**, 191-202 (1999).

103. Goutier, W., Lowry, J.P., McCreary, A.C. & O'Connor, J.J. Frequency-Dependent Modulation of Dopamine Release by Nicotine and Dopamine D1 Receptor Ligands: An In Vitro Fast Cyclic Voltammetry Study in Rat Striatum. *Neurochemical research* **41**, 945-950 (2016).

104. Trulson, M.E. Simultaneous recording of dorsal raphe unit activity and serotonin release in the striatum using voltammetry in awake, behaving cats. *Life sciences* **37**, 2199-2204 (1985).

105. O'Connor, J.J. & Kruk, Z.L. Frequency dependence of 5-HT autoreceptor function in rat dorsal raphe and suprachiasmatic nuclei studied using fast cyclic voltammetry. *Brain research* **568**, 123-130 (1991).

106. Zhang, Z. et al. Release mode of large and small dense-core vesicles specified by different synaptotagmin isoforms in PC12 cells. *Molecular biology of the cell* **22**, 2324-2336 (2011).

107. Barg, S. et al. Delay between fusion pore opening and peptide release from large dense-core vesicles in neuroendocrine cells. *Neuron* **33**, 287-299 (2002).

108. Bruns, D. & Jahn, R. Monoamine transmitter release from small synaptic and large dense-core vesicles. *Advances in pharmacology* **42**, 87-90 (1998).

109. Hokfelt, T. et al. Coexistence of peptides and putative transmitters in neurons. *Advances in biochemical psychopharmacology* **22**, 1-23 (1980).

110. Hokfelt, T. et al. Evidence for coexistence of dopamine and CCK in meso-limbic neurones. *Nature* **285**, 476-478 (1980).

111. Lundberg, J.M. et al. Coexistence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamine in some peripheral and central neurons. *Acta physiologica Scandinavica* **110**, 107-109 (1980).

112. Walker, Q.D., Rooney, M.B., Wightman, R.M. & Kuhn, C.M. Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. *Neuroscience* **95**, 1061-1070 (2000).

113. Palij, P. et al. Presynaptic regulation of dopamine release in corpus striatum monitored in vitro in real time by fast cyclic voltammetry. *Brain research* **509**, 172-174 (1990).

114. May, L.J. & Wightman, R.M. Heterogeneity of stimulated dopamine overflow within rat striatum as observed with in vivo voltammetry. *Brain research* **487**, 311-320 (1989).

115. Gonon, F., Buda, M., Cespuglio, R., Jouvet, M. & Pujol, J.F. Voltammetry in the striatum of chronic freely moving rats: detection of catechols and ascorbic acid. *Brain research* **223**, 69-80 (1981).

116. Gonon, F.G. & Buda, M.J. Regulation of Dopamine Release by Impulse Flow and by Autoreceptors as Studied by Invivo Voltammetry in the Rat Striatum. *Neuroscience* **14**, 765-774 (1985).

117. Tessmar-Raible, K. et al. Conserved sensory-neurosecretory cell types in annelid and fish forebrain: insights into hypothalamus evolution. *Cell* **129**, 1389-1400 (2007).

118. Watts, A.G. 60 YEARS OF NEUROENDOCRINOLOGY: The structure of the neuroendocrine hypothalamus: the neuroanatomical legacy of Geoffrey Harris. *The Journal of endocrinology* **226**, T25-39 (2015).

119. Brock, M. The hypothalamus: new ideas on an old structure. *Acta neurochirurgica. Supplementum* **47**, 127-128 (1990).

120. Demski, L.S., Evan, A.P. & Saland, L.C. The structure of the inferior lobe of the teleost hypothalamus. *The Journal of comparative neurology* **161**, 483-497 (1975).

121. Lammers, H.J. & Lohman, A.H. Structure and fiber connections of the hypothalamus in mammals. *Progress in brain research* **41**, 61-78 (1974).

122. Kaprara, A. & Huhtaniemi, I.T. The hypothalamus-pituitary-gonad axis: Tales of mice and men. *Metabolism: clinical and experimental* **86**, 3-17 (2018).

123. Mebis, L. & van den Berghe, G. The hypothalamus-pituitary-thyroid axis in critical illness. *The Netherlands journal of medicine* **67**, 332-340 (2009).

124. DeMorrow, S. Role of the Hypothalamic-Pituitary-Adrenal Axis in Health and Disease. *International journal of molecular sciences* **19** (2018).

125. Hanley, N.R. & Van de Kar, L.D. Serotonin and the neuroendocrine regulation of the hypothalamic--pituitary-adrenal axis in health and disease. *Vitam Horm* **66**, 189-255 (2003).

126. Baylis, P.H. Posterior pituitary function in health and disease. *Clinics in endocrinology and metabolism* **12**, 747-770 (1983).

127. Lim, C.T. & Khoo, B. in Endotext. (eds. L.J. De Groot et al.) (South Dartmouth (MA); 2000).

128. Sam, S. & Frohman, L.A. Normal physiology of hypothalamic pituitary regulation. *Endocrinol Metab Clin North Am* **37**, 1-22, vii (2008).

129. Annunziato, L., Di Renzo, G., Amoroso, S. & Quattrone, A. Release of endogenous dopamine from tuberoinfundibular neurons. *Life sciences* **35**, 399-407 (1984).

130. Gudelsky, G.A. Tuberoinfundibular dopamine neurons and the regulation of prolactin secretion. *Psychoneuroendocrinology* **6**, 3-16 (1981).

131. Gudelsky, G.A. & Porter, J.C. Release of dopamine from tuberoinfundibular neurons into pituitary stalk blood after prolactin or haloperidol administration. *Endocrinology* **106**, 526-529 (1980).

132. Demarest, K.T. & Moore, K.E. Comparison of dopamine synthesis regulation in the terminals of nigrostriatal, mesolimbic, tuberoinfundibular and tuberohypophyseal neurons. *Journal of neural transmission* **46**, 263-277 (1979).

133. Moore, K.E., Annunziato, L. & Gudelsky, G.A. Studies on tuberoinfundibular dopamine neurons. *Advances in biochemical psychopharmacology* **19**, 193-204 (1978).

134. Fuxe, K. Cellular Localization of Monoamines in the Median Eminence and the Infundibular Stem of Some Mammals. *Zeitschrift fur Zellforschung und mikroskopische Anatomie* **61**, 710-724 (1964).

135. Fuxe, K. Cellular Localization of Monoamines in the Median Eminence and in the Infundibular Stem of Some Mammals. *Acta physiologica Scandinavica* **58**, 383-384 (1963).

136. Kucka, M., Kretschmannova, K., Stojilkovic, S.S., Zemkova, H. & Tomic, M. Dependence of spontaneous electrical activity and basal prolactin release on

nonselective cation channels in pituitary lactotrophs. *Physiological research / Academia Scientiarum Bohemoslovaca* **61**, 267-275 (2012).

137. Gonzalez-Iglesias, A.E., Murano, T., Li, S., Tomic, M. & Stojilkovic, S.S. Dopamine inhibits basal prolactin release in pituitary lactotrophs through pertussis toxin-sensitive and -insensitive signaling pathways. *Endocrinology* **149**, 1470-1479 (2008).

138. Carmeliet, P., Maertens, P. & Denef, C. Stimulation and inhibition of prolactin release from rat pituitary lactotrophs by the cholinomimetic carbachol in vitro. Influence of hormonal environment and intercellular contacts. *Molecular and cellular endocrinology* **63**, 121-131 (1989).

139. Ross, P.C. & Burkman, A.M. Inhibition of prolactin release from anterior pituitary lactotrophs in culture by sulfur-containing analogs of dopamine. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine* **188**, 87-91 (1988).

140. Aguilera, G., Hyde, C.L. & Catt, K.J. Angiotensin II receptors and prolactin release in pituitary lactotrophs. *Endocrinology* **111**, 1045-1050 (1982).

141. Krulich, L., Coppings, R.J., McCann, S.M. & Mayfield, M.A. Inhibition of prolactin secretion by a direct effect of methysergide on the pituitary lactotrophs in the rat. *Life sciences* **23**, 1665-1673 (1978).

142. Campino, C. et al. Bioactivity of prolactin isoforms: lactation and recovery of menses in nursing women. *Hum Reprod* **14**, 898-905 (1999).

143. Dearlove, J.C. & Dearlove, B.M. Prolactin, fluid balance and lactation. *British journal of obstetrics and gynaecology* **88**, 652-654 (1981).

144. Hooley, R.D., Campbell, J.J. & Findlay, J.K. The importance of prolactin for lactation in the ewe. *The Journal of endocrinology* **79**, 301-310 (1978).

145. Smith, M.S. The relative contribution of suckling and prolactin to the inhibition of gonadotropin secretion during lactation in the rat. *Biology of reproduction* **19**, 77-83 (1978).

146. Delvoye, P., Demaegd, M. & Delogne-Desnoeck, J. The influence of the frequency of nursing and of previous lactation experience on serum prolactin in lactating mothers. *J Biosoc Sci* **9**, 447-451 (1977).

147. Prolactin, pregnancy, and lactation. *Br Med J* **4**, 188 (1975).

148. McNeilly, A.S. Lactation and the physiology of prolactin secretion. *Postgraduate medical journal* **51**, 231-235 (1975).

149. Friesen, H.G., Fournier, P. & Desjardins, P. Pituitary prolactin in pregnancy and normal and abnormal lactation. *Clinical obstetrics and gynecology* **16**, 25-45 (1973).

150. Ota, K., Shinde, Y. & Yokoyama, A. Relationship between Oxytocin and Prolactin Secretion in Maintenance of Lactation in Rats. *Endocrinology* **76**, 1-8 (1965).

151. Meites, J., Hopkins, T.F. & Talwalker, P.K. Induction of Lactation in Pregnant Rabbits with Prolactin, Cortisol Acetate or Both. *Endocrinology* **73**, 261-264 (1963).

152. Meites, J. & Shelesnyak, M.C. Effects of prolactin on duration of pregnancy, viability of young and lactation in rats. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine* **94**, 746-749 (1957).

153. Binart, N. et al. Male reproductive function is not affected in prolactin receptor-deficient mice. *Endocrinology* **144**, 3779-3782 (2003).

154. Carani, C., Granata, A.R., Fustini, M.F. & Marrama, P. Prolactin and testosterone: their role in male sexual function. *International journal of andrology* **19**, 48-54 (1996).

155. Rao, A.J. & Kotagi, S.G. Effect of suppression of prolactin on gonadal function in immature male hamsters. *Andrologia* **21**, 498-501 (1989).

156. Shiota, K., Takahashi, M. & Suzuki, Y. Testicular function of actively immunized male rats with LH releasing hormone (LHRH): a possible role of prolactin on regulation of spermatogenesis. *Endocrinologia japonica* **28**, 521-534 (1981).

157. Boes, A.D. et al. Connectivity of sleep- and wake-promoting regions of the human hypothalamus observed during resting wakefulness. *Sleep* **41** (2018).

158. Sharma, R., Sahota, P. & Thakkar, M.M. Melatonin promotes sleep in mice by inhibiting orexin neurons in the perifornical lateral hypothalamus. *Journal of pineal research* **65**, e12498 (2018).

159. Sapin, E. et al. A very large number of GABAergic neurons are activated in the tuberal hypothalamus during paradoxical (REM) sleep hypersomnia. *PloS one* **5**, e11766 (2010).

160. Hassani, O.K., Henny, P., Lee, M.G. & Jones, B.E. GABAergic neurons intermingled with orexin and MCH neurons in the lateral hypothalamus discharge maximally during sleep. *The European journal of neuroscience* **32**, 448-457 (2010).

161. Peterfi, Z., Makara, G.B., Obal, F., Jr. & Krueger, J.M. The anterolateral projections of the medial basal hypothalamus affect sleep. *American journal of physiology. Regulatory, integrative and comparative physiology* **296**, R1228-1238 (2009).

162. Suntsova, N. et al. The median preoptic nucleus reciprocally modulates activity of arousal-related and sleep-related neurons in the perifornical lateral hypothalamus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 1616-1630 (2007).

163. Mohns, E.J., Karlsson, K.A. & Blumberg, M.S. The preoptic hypothalamus and basal forebrain play opposing roles in the descending modulation of sleep and wakefulness in infant rats. *The European journal of neuroscience* **23**, 1301-1310 (2006).

164. Gerashchenko, D. & Shiromani, P.J. Different neuronal phenotypes in the lateral hypothalamus and their role in sleep and wakefulness. *Molecular neurobiology* **29**, 41-59 (2004).

165. Mignot, E., Taheri, S. & Nishino, S. Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. *Nature neuroscience* **5 Suppl**, 1071-1075 (2002).

166. Suntsova, N.V., Dergacheva, O.Y. & Burikov, A.A. The role of the posterior hypothalamus in controlling the paradoxical phase of sleep. *Neuroscience and behavioral physiology* **30**, 161-167 (2000).

167. Steininger, T.L., Alam, M.N., Gong, H., Szymusiak, R. & McGinty, D. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. *Brain research* **840**, 138-147 (1999).

168. Nitz, D. & Siegel, J.M. GABA release in posterior hypothalamus across sleep-wake cycle. *The American journal of physiology* **271**, R1707-1712 (1996).

169.   Freemon, F.R., Agnew, H.W., Jr. & Wilder, B.J. Electrical activity of the human hypothalamus and midbrain during sleep. *Comprehensive psychiatry* **11**, 356-360 (1970).

170.   Findlay, A.L. & Hayward, J.N. Spontaneous activity of single neurones in the hypothalamus of rabbits during sleep and waking. *The Journal of physiology* **201**, 237-258 (1969).

171.   Cruz-Martinez, A.M. et al. CB1 receptors in the paraventricular nucleus of the hypothalamus modulate the release of 5-HT and GABA to stimulate food intake in rats. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* (2018).

172.   Jeong, J.H., Lee, D.K. & Jo, Y.H. Cholinergic neurons in the dorsomedial hypothalamus regulate food intake. *Molecular metabolism* **6**, 306-312 (2017).

173.   Chen, Y.W., Morganstern, I., Barson, J.R., Hoebel, B.G. & Leibowitz, S.F. Differential role of D1 and D2 receptors in the perifornical lateral hypothalamus in controlling ethanol drinking and food intake: possible interaction with local orexin neurons. *Alcoholism, clinical and experimental research* **38**, 777-786 (2014).

174.   Fedeli, A. et al. The paraventricular nucleus of the hypothalamus is a neuroanatomical substrate for the inhibition of palatable food intake by neuropeptide S. *The European journal of neuroscience* **30**, 1594-1602 (2009).

175.   Meister, B. Control of food intake via leptin receptors in the hypothalamus. *Vitam Horm* **59**, 265-304 (2000).

176.   Laviano, A. et al. Serotoninergic block in the ventromedial nucleus of hypothalamus improves food intake in anorectic tumor bearing rats. *Advances in experimental medicine and biology* **398**, 551-553 (1996).

177.   Ookuma, K. et al. Neuronal histamine in the hypothalamus suppresses food intake in rats. *Brain research* **628**, 235-242 (1993).

178.   Debons, A.F. & Krimsky, I. Regulation of food intake: role of the ventromedial hypothalamus. *Postgraduate medicine* **51**, 74-78 (1972).

179.   Ingram, D.L. Effects of heating and cooling the hypothalamus on food intake in the pig. *Brain research* **11**, 714-716 (1968).

180.   Morrison, S.D. & Mayer, J. Effect of sham operations in the hypothalamus on food and water intake of the rat. *The American journal of physiology* **191**, 255-258 (1957).

181.   Mayer, J., Bates, M.W. & Van Itallie, T.B. Blood sugar and food intake in rats with lesions of the anterior hypothalamus. *Metabolism: clinical and experimental* **1**, 340-348 (1952).

182.   Kuroda, K.O. & Numan, M. The medial preoptic area and the regulation of parental behavior. *Neuroscience bulletin* **30**, 863-865 (2014).

183.   Lee, A.W. & Brown, R.E. Comparison of medial preoptic, amygdala, and nucleus accumbens lesions on parental behavior in California mice (Peromyscus californicus). *Physiology & behavior* **92**, 617-628 (2007).

184.   Lee, A.W. & Brown, R.E. Medial preoptic lesions disrupt parental behavior in both male and female California mice (Peromyscus californicus). *Behavioral neuroscience* **116**, 968-975 (2002).

185. Wellman, J. et al. Preoptic area infusions of morphine disrupt--and naloxone restores--parental-like behavior in juvenile rats. *Brain research bulletin* **44**, 183-191 (1997).

186. Slawski, B.A. & Buntin, J.D. Preoptic area lesions disrupt prolactin-induced parental feeding behavior in ring doves. *Hormones and behavior* **29**, 248-266 (1995).

187. Hashikawa, Y., Hashikawa, K., Falkner, A.L. & Lin, D. Ventromedial Hypothalamus and the Generation of Aggression. *Frontiers in systems neuroscience* **11**, 94 (2017).

188. Yang, T. et al. Social Control of Hypothalamus-Mediated Male Aggression. *Neuron* **95**, 955-970 e954 (2017).

189. Goodson, J.L., Kelly, A.M., Kingsbury, M.A. & Thompson, R.R. An aggression-specific cell type in the anterior hypothalamus of finches. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 13847-13852 (2012).

190. Lin, D. et al. Functional identification of an aggression locus in the mouse hypothalamus. *Nature* **470**, 221-226 (2011).

191. Spiteri, T. et al. The role of the estrogen receptor alpha in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety and aggression. *Behavioural brain research* **210**, 211-220 (2010).

192. Kabelik, D., Crombie, T. & Moore, M.C. Aggression frequency and intensity, independent of testosterone levels, relate to neural activation within the dorsolateral subdivision of the ventromedial hypothalamus in the tree lizard Urosaurus ornatus. *Hormones and behavior* **54**, 18-27 (2008).

193. Kruk, M.R. et al. Discriminant analysis of the localization of aggression-inducing electrode placements in the hypothalamus of male rats. *Brain research* **260**, 61-79 (1983).

194. Panksepp, J. Aggression elicited by electrical stimulation of the hypothalamus in albino rats. *Physiology & behavior* **6**, 321-329 (1971).

195. Kohl, J. & Dulac, C. Neural control of parental behaviors. *Current opinion in neurobiology* **49**, 116-122 (2018).

196. Pilakouta, N., Hanlon, E.J.H. & Smiseth, P.T. Biparental care is more than the sum of its parts: experimental evidence for synergistic effects on offspring fitness. *Proceedings. Biological sciences / The Royal Society* **285** (2018).

197. Saltzman, W. et al. Effects of Parental Status on Male Body Mass in the Monogamous, Biparental California Mouse. *J Zool (1987)* **296**, 23-29 (2015).

198. Mattey, S.N. & Smiseth, P.T. Complex effects of inbreeding on biparental cooperation. *The American naturalist* **185**, 1-12 (2015).

199. McInroy, J.K., Brousmiche, D.G. & Wynne-Edwards, K.E. Fathers, fat, and maternal energetics in a biparental hamster: paternal presence determines the outcome of a current reproductive effort and adipose tissue limits subsequent reproductive effort. *Hormones and behavior* **37**, 399-409 (2000).

200. Runcie, M.J. Biparental care and obligate monogamy in the rock-haunting possum, Petropseudes dahli, from tropical Australia. *Animal behaviour* **59**, 1001-1008 (2000).

201. Reburn, C.J. & Wynne-Edwards, K.E. Hormonal changes in males of a naturally biparental and a uniparental mammal. *Hormones and behavior* **35**, 163-176 (1999).

202. Wu, Z., Autry, A.E., Bergan, J.F., Watabe-Uchida, M. & Dulac, C.G. Galanin neurons in the medial preoptic area govern parental behaviour. *Nature* **509**, 325-330 (2014).

203. Brouwer, L., van de Pol, M. & Cockburn, A. The role of social environment on parental care: offspring benefit more from the presence of female than male helpers. *The Journal of animal ecology* **83**, 491-503 (2014).

204. Huang, W.S. & Pike, D.A. Testing cost-benefit models of parental care evolution using lizard populations differing in the expression of maternal care. *PloS one* **8**, e54065 (2013).

205. Bendesky, A. et al. The genetic basis of parental care evolution in monogamous mice. *Nature* **544**, 434-439 (2017).

206. Scott, N., Prigge, M., Yizhar, O. & Kimchi, T. A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion. *Nature* **525**, 519-522 (2015).

207. Brown, R.S., Herbison, A.E. & Grattan, D.R. Differential changes in responses of hypothalamic and brainstem neuronal populations to prolactin during lactation in the mouse. *Biology of reproduction* **84**, 826-836 (2011).

208. Luckman, S.M. Fos expression within regions of the preoptic area, hypothalamus and brainstem during pregnancy and parturition. *Brain research* **669**, 115-124 (1995).

209. Brown, R.S.E. et al. Prolactin action in the medial preoptic area is necessary for postpartum maternal nursing behavior. *Proceedings of the National Academy of Sciences of the United States of America* **114**, 10779-10784 (2017).

210. Akther, S., Fakhrul, A.A. & Higashida, H. Effects of electrical lesions of the medial preoptic area and the ventral pallidum on mate-dependent paternal behavior in mice. *Neuroscience letters* **570**, 21-25 (2014).

211. Kuroda, K.O. et al. ERK-FosB signaling in dorsal MPOA neurons plays a major role in the initiation of parental behavior in mice. *Molecular and cellular neurosciences* **36**, 121-131 (2007).

212. Oxley, G. & Fleming, A.S. The effects of medial preoptic area and amygdala lesions on maternal behavior in the juvenile rat. *Developmental psychobiology* **37**, 253-265 (2000).

213. Buntin, J.D., Halawani, M.E., Ottinger, M.A., Fan, Y. & Fivizzani, A.J. An analysis of sex and breeding stage differences in prolactin binding activity in brain and hypothalamic GnRH concentration in Wilson's phalarope, a sex role-reversed species. *General and comparative endocrinology* **109**, 119-132 (1998).

214. Kirkpatrick, B., Kim, J.W. & Insel, T.R. Limbic system fos expression associated with paternal behavior. *Brain research* **658**, 112-118 (1994).

215. Barrett, J. et al. Maternal affect and quality of parenting experiences are related to amygdala response to infant faces. *Social neuroscience* **7**, 252-268 (2012).

216. Kramer, K.M., Carr, M.S., Schmidt, J.V. & Cushing, B.S. Parental regulation of central patterns of estrogen receptor alpha. *Neuroscience* **142**, 165-173 (2006).

217. Caldji, C., Diorio, J., Anisman, H. & Meaney, M.J. Maternal behavior regulates benzodiazepine/GABAA receptor subunit expression in brain regions associated with fear in BALB/c and C57BL/6 mice. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **29**, 1344-1352 (2004).

218. Bester-Meredith, J.K., Young, L.J. & Marler, C.A. Species differences in paternal behavior and aggression in peromyscus and their associations with vasopressin immunoreactivity and receptors. *Hormones and behavior* **36**, 25-38 (1999).

219. Woodworth, C.H. Attack elicited in rats by electrical stimulation of the lateral hypothalamus. *Physiology & behavior* **6**, 345-353 (1971).

220. Sano, K., Mayanagi, Y., Sekino, H., Ogashiwa, M. & Ishijima, B. Results of stimulation and destruction of the posterior hypothalamus in man. *Journal of neurosurgery* **33**, 689-707 (1970).

221. Siegel, A. & Skog, D. Effects of electrical stimulation of the septum upon attack behavior elicited from the hypothalamus in the cat. *Brain research* **23**, 371-380 (1970).

222. Bandler, R.J., Jr. Facilitation of aggressive behaviour in rat by direct cholinergic stimulation of the hypothalamus. *Nature* **224**, 1035-1036 (1969).

223. Flynn, J.P. Neural aspects of attack behavior in cats. *Annals of the New York Academy of Sciences* **159**, 1008-1012 (1969).

224. Roberts, W.W. & Bergquist, E.H. Attack elicited by hypothalamic stimulation in cats raised in social isolation. *Journal of comparative and physiological psychology* **66**, 590-595 (1968).

225. MacDonnell, M. & Flynn, J.P. Sensory control of hypothalamic attack. *Animal behaviour* **14**, 399-405 (1966).

226. McAdam, D.W. & Kaelber, W.W. Differential impairment of avoidance learning in cats with ventromedial hypothalamic lesions. *Experimental neurology* **15**, 293-298 (1966).

227. Putkonen, P.T. Attack elicited by forebrain and hypothalamic stimulation in the chicken. *Experientia* **22**, 405-407 (1966).

228. Sano, K., Yoshioka, M., Ogashiwa, M., Ishijima, B. & Ohye, C. Postero-medial hypothalamotomy in the treatment of aggressive behaviors. *Confinia neurologica* **27**, 164-167 (1966).

229. Roberts, W.W. & Kiess, H.O. Motivational Properties of Hypothalamic Aggression in Cats. *Journal of comparative and physiological psychology* **58**, 187-193 (1964).

230. Karli, P. [Septum, posterior hypothalamus and interspecific rat-mouse aggressiveness]. *Journal de physiologie* **52**, 135-136 (1960).

231. Karli, P. [Effects of experimental lesions of the mammillary nodes on rat-mouse interspecies aggression]. *Comptes rendus des seances de la Societe de biologie et de ses filiales* **154**, 1287-1290 (1960).

232. Wong, L.C. et al. Effective Modulation of Male Aggression through Lateral Septum to Medial Hypothalamus Projection. *Current biology : CB* **26**, 593-604 (2016).

233. McDonald, M.M., Markham, C.M., Norvelle, A., Albers, H.E. & Huhman, K.L. GABAA receptor activation in the lateral septum reduces the expression of conditioned defeat and increases aggression in Syrian hamsters. *Brain research* **1439**, 27-33 (2012).

234. Lee, G. & Gammie, S.C. GABA(A) receptor signaling in the lateral septum regulates maternal aggression in mice. *Behavioral neuroscience* **123**, 1169-1177 (2009).

235. Levinson, D.M., Reeves, D.L. & Buchanan, D.R. Reductions in aggression and dominance status in guinea pigs following bilateral lesions in the basolateral amygdala or lateral septum. *Physiology & behavior* **25**, 963-971 (1980).

236. Albert, D.J. & Richmond, S.E. Reactivity and aggression in the rat: induction by alpha-adrenergic blocking agents injected ventral to anterior septum but not into lateral septum. *Journal of comparative and physiological psychology* **91**, 886-896 (1977).

237. Choy, O., Raine, A. & Hamilton, R.H. Stimulation of the Prefrontal Cortex Reduces Intentions to Commit Aggression: A Randomized, Double-Blind, Placebo-Controlled, Stratified, Parallel-Group Trial. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **38**, 6505-6512 (2018).

238. Sabihi, S., Dong, S.M., Durosko, N.E. & Leuner, B. Oxytocin in the medial prefrontal cortex regulates maternal care, maternal aggression and anxiety during the postpartum period. *Frontiers in behavioral neuroscience* **8**, 258 (2014).

239. Takahashi, A., Nagayasu, K., Nishitani, N., Kaneko, S. & Koide, T. Control of intermale aggression by medial prefrontal cortex activation in the mouse. *PloS one* **9**, e94657 (2014).

240. Blair, R.J. Psychopathy, frustration, and reactive aggression: the role of ventromedial prefrontal cortex. *Br J Psychol* **101**, 383-399 (2010).

241. Lotze, M., Veit, R., Anders, S. & Birbaumer, N. Evidence for a different role of the ventral and dorsal medial prefrontal cortex for social reactive aggression: An interactive fMRI study. *NeuroImage* **34**, 470-478 (2007).

242. New, A.S. et al. Fluoxetine increases relative metabolic rate in prefrontal cortex in impulsive aggression. *Psychopharmacology* **176**, 451-458 (2004).

243. da Cunha-Bang, S., Fisher, P.M., Hjordt, L.V., Holst, K. & Knudsen, G.M. Amygdala reactivity to fearful faces correlates positively with impulsive aggression. *Social neuroscience*, 1-11 (2018).

244. Pardini, D.A., Raine, A., Erickson, K. & Loeber, R. Lower amygdala volume in men is associated with childhood aggression, early psychopathic traits, and future violence. *Biological psychiatry* **75**, 73-80 (2014).

245. Matthies, S. et al. Small amygdala-high aggression? The role of the amygdala in modulating aggression in healthy subjects. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry* **13**, 75-81 (2012).

246. Suzuki, H., Han, S.D. & Lucas, L.R. Increased 5-HT1B receptor density in the basolateral amygdala of passive observer rats exposed to aggression. *Brain research bulletin* **83**, 38-43 (2010).

247. Bosch, O.J. & Neumann, I.D. Vasopressin released within the central amygdala promotes maternal aggression. *The European journal of neuroscience* **31**, 883-891 (2010).

248. Ferris, C.F. et al. Oxytocin in the amygdala facilitates maternal aggression. *Annals of the New York Academy of Sciences* **652**, 456-457 (1992).

249. Halasz, J., Liposits, Z., Meelis, W., Kruk, M.R. & Haller, J. Hypothalamic attack area-mediated activation of the forebrain in aggression. *Neuroreport* **13**, 1267-1270 (2002).

250. Haller, J. et al. Aggressive experience affects the sensitivity of neurons towards pharmacological treatment in the hypothalamic attack area. *Behavioural pharmacology* **9**, 469-475 (1998).

251. Bermond, B., Mos, J., Meelis, W., van der Poel, A.M. & Kruk, M.R. Aggression induced by stimulation of the hypothalamus: effects of androgens. *Pharmacology, biochemistry, and behavior* **16**, 41-45 (1982).

252. Kruk, M.R. Hypothalamic attack: a wonderful artifact or a useful perspective on escalation and pathology in aggression? A viewpoint. *Current topics in behavioral neurosciences* **17**, 143-188 (2014).

253. Kruk, M.R. Ethology and pharmacology of hypothalamic aggression in the rat. *Neuroscience and biobehavioral reviews* **15**, 527-538 (1991).

254. Lammers, J.H., Kruk, M.R., Meelis, W. & van der Poel, A.M. Hypothalamic substrates for brain stimulation-induced attack, teeth-chattering and social grooming in the rat. *Brain research* **449**, 311-327 (1988).

255. Kruk, M.R. et al. Comparison of aggressive behaviour induced by electrical stimulation in the hypothalamus of male and female rats. *Progress in brain research* **61**, 303-314 (1984).

256. Kruk, M.R. et al. Brain-stimulation induced agonistic behaviour: a novel paradigm in ethopharmacological aggression research. *Progress in clinical and biological research* **167**, 157-177 (1984).

257. Kruk, M.R. & van der Poel, A.M. Is there evidence for a neural correlate of an aggressive behavioural system in the hypothalamus of the rat? *Progress in brain research* **53**, 385-390 (1980).

258. Kruk, M.R., van der Poel, A.M. & de Vos-Frerichs, T.P. The induction of aggressive behaviour by electrical stimulation in the hypothalamus of male rats. *Behaviour* **70**, 292-322 (1979).

259. Falkner, A.L., Grosenick, L., Davidson, T.J., Deisseroth, K. & Lin, D. Hypothalamic control of male aggression-seeking behavior. *Nature neuroscience* **19**, 596-604 (2016).

260. Yu, Q. et al. Optogenetic stimulation of DAergic VTA neurons increases aggression. *Molecular psychiatry* **19**, 635 (2014).

261. Lee, H. et al. Scalable control of mounting and attack by Esr1+ neurons in the ventromedial hypothalamus. *Nature* **509**, 627-632 (2014).

262. Nieh, E.H., Kim, S.Y., Namburi, P. & Tye, K.M. Optogenetic dissection of neural circuits underlying emotional valence and motivated behaviors. *Brain research* **1511**, 73-92 (2013).

263. Potts, J.R. & Lewis, M.A. How memory of direct animal interactions can lead to territorial pattern formation. *Journal of the Royal Society, Interface* **13** (2016).

264. Pruitt, J.N. & Pinter-Wollman, N. The legacy effects of keystone individuals on collective behaviour scale to how long they remain within a group. *Proceedings. Biological sciences / The Royal Society* **282** (2015).

265. Gan, G. et al. Reward vs. Retaliation-the Role of the Mesocorticolimbic Salience Network in Human Reactive Aggression. *Frontiers in behavioral neuroscience* **10**, 179 (2016).

266. Golden, S.A. et al. Basal forebrain projections to the lateral habenula modulate aggression reward. *Nature* **534**, 688-692 (2016).

267. Chester, D.S. et al. Looking for reward in all the wrong places: dopamine receptor gene polymorphisms indirectly affect aggression through sensation-seeking. *Social neuroscience* **11**, 487-494 (2016).

268. Chester, D.S. & DeWall, C.N. The pleasure of revenge: retaliatory aggression arises from a neural imbalance toward reward. *Social cognitive and affective neuroscience* **11**, 1173-1182 (2016).

269. Fish, E.W., DeBold, J.F. & Miczek, K.A. Escalated aggression as a reward: corticosterone and GABA(A) receptor positive modulators in mice. *Psychopharmacology* **182**, 116-127 (2005).

270. Barry, J.A. et al. Prolactin and aggression in women with fertility problems. *Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology* **34**, 605-610 (2014).

271. Broida, J., Michael, S.D. & Svare, B. Plasmin prolactin levels are not related to the initiation, maintenance, and decline of postpartum aggression in mice. *Behavioral and neural biology* **32**, 121-125 (1981).

272. Gleason, P.E., Michael, S.D. & Christian, J.J. Brief report prolactin-induced aggression in female Peromyscus leucopus. *Behavioral and neural biology* **33**, 243-248 (1981).

273. New, A.S. et al. Low prolactin response to fenfluramine in impulsive aggression. *Journal of psychiatric research* **38**, 223-230 (2004).

274. Wise, D.A. & Pryor, T.L. Effects of ergocornine and prolactin on aggression in the postpartum golden hamster. *Hormones and behavior* **8**, 30-39 (1977).

275. Tsien, J.Z. Cre-Lox Neurogenetics: 20 Years of Versatile Applications in Brain Research and Counting. *Front Genet* **7**, 19 (2016).

276. Bertram, R., Kolb, M. & Hillen, W. In vivo activation of tetracycline repressor by Cre/lox-mediated gene assembly. *Journal of molecular microbiology and biotechnology* **17**, 136-145 (2009).

277. Yamamoto, M. et al. A multifunctional reporter mouse line for Cre- and FLP-dependent lineage analysis. *Genesis* **47**, 107-114 (2009).

278. Mortensen, R. Overview of gene targeting by homologous recombination. *Current protocols in molecular biology* **Chapter 23**, Unit 23 21 (2006).

279. Liu, P., Jenkins, N.A. & Copeland, N.G. Efficient Cre-loxP-induced mitotic recombination in mouse embryonic stem cells. *Nature genetics* **30**, 66-72 (2002).

280. Akagi, K. et al. Cre-mediated somatic site-specific recombination in mice. *Nucleic acids research* **25**, 1766-1773 (1997).

281. Sauer, B. & Henderson, N. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 5166-5170 (1988).

282. Cox, M.M. The FLP protein of the yeast 2-microns plasmid: expression of a eukaryotic genetic recombination system in Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 4223-4227 (1983).

283. Broach, J.R., Guarascio, V.R. & Jayaram, M. Recombination within the Yeast Plasmid 2-Mu Circle Is Site-Specific. *Cell* **29**, 227-234 (1982).

284. Neher, E. & Sakmann, B. The patch clamp technique. *Scientific American* **266**, 44-51 (1992).

285. Sakmann, B. & Neher, E. Patch clamp techniques for studying ionic channels in excitable membranes. *Annu Rev Physiol* **46**, 455-472 (1984).

286. Hamill, O.P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F.J. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Archiv : European journal of physiology* **391**, 85-100 (1981).

287. Neher, E., Sakmann, B. & Steinbach, J.H. The extracellular patch clamp: a method for resolving currents through individual open channels in biological membranes. *Pflugers Archiv : European journal of physiology* **375**, 219-228 (1978).

288. John, C.E. & Jones, S.R. in Electrochemical Methods for Neuroscience. (eds. A.C. Michael & L.M. Borland) (Boca Raton (FL); 2007).

289. Robinson, D.L. & Wightman, R.M. in Electrochemical Methods for Neuroscience. (eds. A.C. Michael & L.M. Borland) (Boca Raton (FL); 2007).

290. Bass, C.E. et al. Optogenetic control of striatal dopamine release in rats. *Journal of neurochemistry* **114**, 1344-1352 (2010).

291. Ford, C.P., Gantz, S.C., Phillips, P.E. & Williams, J.T. Control of extracellular dopamine at dendrite and axon terminals. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **30**, 6975-6983 (2010).

292. Garris, P.A., Collins, L.B., Jones, S.R. & Wightman, R.M. Evoked extracellular dopamine in vivo in the medial prefrontal cortex. *Journal of neurochemistry* **61**, 637-647 (1993).

293. Zimmerman, J.B. & Wightman, R.M. Simultaneous electrochemical measurements of oxygen and dopamine in vivo. *Analytical chemistry* **63**, 24-28 (1991).

294. Wightman, R.M. et al. Real-time characterization of dopamine overflow and uptake in the rat striatum. *Neuroscience* **25**, 513-523 (1988).

295. Kuhr, W.G., Wightman, R.M. & Rebec, G.V. Dopaminergic neurons: simultaneous measurements of dopamine release and single-unit activity during stimulation of the medial forebrain bundle. *Brain research* **418**, 122-128 (1987).

296. Kuhr, W.G. & Wightman, R.M. Real-time measurement of dopamine release in rat brain. *Brain research* **381**, 168-171 (1986).

297. Mundroff, M.L. & Wightman, R.M. Amperometry and cyclic voltammetry with carbon fiber microelectrodes at single cells. *Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.]* **Chapter 6**, Unit 6 14 (2002).

298. Deisseroth, K. Optogenetics: 10 years of microbial opsins in neuroscience. *Nature neuroscience* **18**, 1213-1225 (2015).

299. Yizhar, O., Fenno, L.E., Davidson, T.J., Mogri, M. & Deisseroth, K. Optogenetics in neural systems. *Neuron* **71**, 9-34 (2011).

300. Franklin, K.B.J. & Paxinos, G. THE MOUSE BRAIN IN STEREOTACTIC COORDINATES, Vol. Third edition. (2008).

301. Cao, W.Y. et al. Role of early environmental enrichment on the social dominance tube test at adulthood in the rat. *Psychopharmacology* **234**, 3321-3334 (2017).

302. Wang, F., Kessels, H.W. & Hu, H. The mouse that roared: neural mechanisms of social hierarchy. *Trends in neurosciences* **37**, 674-682 (2014).

303. Robinson, J.C., Chapman, C.A. & Courtemanche, R. Gap Junction Modulation of Low-Frequency Oscillations in the Cerebellar Granule Cell Layer. *Cerebellum* **16**, 802-811 (2017).

304. Shimizu, K. & Stopfer, M. Gap junctions. *Current biology : CB* **23**, R1026-1031 (2013).

305. Posluszny, A. The contribution of electrical synapses to field potential oscillations in the hippocampal formation. *Frontiers in neural circuits* **8**, 32 (2014).

306. Coulon, P. & Landisman, C.E. The Potential Role of Gap Junctional Plasticity in the Regulation of State. *Neuron* **93**, 1275-1295 (2017).

307. De Zeeuw, C.I., Hoebeek, F.E. & Schonewille, M. Causes and consequences of oscillations in the cerebellar cortex. *Neuron* **58**, 655-658 (2008).

308. Bass, C.E., Grinevich, V.P., Kulikova, A.D., Bonin, K.D. & Budygin, E.A. Terminal effects of optogenetic stimulation on dopamine dynamics in rat striatum. *Journal of neuroscience methods* **214**, 149-155 (2013).

309. Bass, C.E. et al. Optogenetic control of striatal dopamine release in rats. *Journal of neurochemistry* **114**, 1344-1352 (2010).

310. Rosenblatt, J.S., Hazelwood, S. & Poole, J. Maternal behavior in male rats: effects of medial preoptic area lesions and presence of maternal aggression. *Hormones and behavior* **30**, 201-215 (1996).

311. Sakaguchi, K. et al. Induction of brain prolactin receptor long-form mRNA expression and maternal behavior in pup-contacted male rats: promotion by prolactin administration and suppression by female contact. *Neuroendocrinology* **63**, 559-568 (1996).

312. Motta, S.C. et al. Ventral premammillary nucleus as a critical sensory relay to the maternal aggression network. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 14438-14443 (2013).

313. Gubernick, D.J. & Nelson, R.J. Prolactin and paternal behavior in the biparental California mouse, Peromyscus californicus. *Hormones and behavior* **23**, 203-210 (1989).

314. Gubernick, D.J. & Alberts, J.R. The biparental care system of the California mouse, Peromyscus californicus. *Journal of comparative psychology* **101**, 169-177 (1987).

315. Lim, M.M., Nair, H.P. & Young, L.J. Species and sex differences in brain distribution of corticotropin-releasing factor receptor subtypes 1 and 2 in monogamous and promiscuous vole species. *The Journal of comparative neurology* **487**, 75-92 (2005).

316. Wang, Z., Young, L.J., Liu, Y. & Insel, T.R. Species differences in vasopressin receptor binding are evident early in development: comparative anatomic studies in prairie and montane voles. *The Journal of comparative neurology* **378**, 535-546 (1997).

317. Shapiro, L.E. & Dewsbury, D.A. Differences in affiliative behavior, pair bonding, and vaginal cytology in two species of vole (Microtus ochrogaster and M. montanus). *Journal of comparative psychology* **104**, 268-274 (1990).

318. Shapiro, L.E. & Insel, T.R. Infant's response to social separation reflects adult differences in affiliative behavior: a comparative developmental study in prairie and montane voles. *Developmental psychobiology* **23**, 375-393 (1990).

319. Sacchi, R. et al. Seasonal variations of plasma testosterone among colour-morph common wall lizards (Podarcis muralis). *General and comparative endocrinology* **240**, 114-120 (2017).

320. Palagi, E. & Norscia, I. The Season for Peace: Reconciliation in a Despotic Species (Lemur catta). *PloS one* **10**, e0142150 (2015).

321. Ostner, J., Heistermann, M. & Schulke, O. Male competition and its hormonal correlates in Assamese macaques (Macaca assamensis). *Hormones and behavior* **59**, 105-113 (2011).

322. Girard-Buttoz, C., Heistermann, M., Krummel, S. & Engelhardt, A. Seasonal and social influences on fecal androgen and glucocorticoid excretion in wild male long-tailed macaques (Macaca fascicularis). *Physiology & behavior* **98**, 168-175 (2009).

323. Kellam, J.S., Wingfield, J.C. & Lucas, J.R. Nonbreeding season pairing behavior and the annual cycle of testosterone in male and female downy woodpeckers, Picoides pubescens. *Hormones and behavior* **46**, 703-714 (2004).

324. Rowell, T.E. & Dixson, A.F. Changes in social organization during the breeding season of wild talapoin monkeys. *Journal of reproduction and fertility* **43**, 419-434 (1975).