Untangling the neurobiology of escalated aggression in animals

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Abstract

Canines' capacity for uncontrolled aggressiveness and violent-like behavior is a serious veterinary medicine concern and inflicts an awful burden on their owners. Unfortunately, the current intervention strategies and treatment options for curbing these problematic behavioral expressions are largely inadequate. Hence, a more fundamental knowledge about the neurobiological determinants of aggression is urgently needed. In particular, the interaction between environmental factors and the neurochemical substrates that causally underlies the shift towards escalated and maladaptive forms of aggressive behavior (e.g., violence) is in great need to be unraveled. Novel experimental laboratory models of violent-like aggression in rodents combined with newly emerging technologies for mapping and manipulating neuronal activity with anatomical, genetic and temporal precision are indispensable to obtain this goal. This contribution presents some of the most significant developments made during the last decade in this understudied preclinical animal research field that promise to significantly advance our understanding of the etiology, brain mechanisms and potential therapeutic interventions of excessive aggressive behaviors.

Key words

Aggression, violence, neurobiology, animal models, serotonin, 5-HT_{1A} autoreceptors, oxytocin, vasopressin.

Introduction.

It is commonly accepted in biology that, throughout the animal kingdom, aggression is one of the most widespread and functional forms of social behavior that ultimately contributes to fitness (procreation) and survival of individuals. Clearly, aggression is the behavioral weapon of choice for essentially all animals and humans to gain and maintain access to desired resources (food, shelter, mates), defend themselves and their offspring from rivals and predators, and establish and secure social status/hierarchical relationships. In both animals and humans, most individuals engage in social conflicts with appropriate and well-controlled (functional) forms of aggressive behavior; only a relatively small percent of individuals may show persistent excessively aggressive behavior or can become extremely violent. This small percentage (ranging from 3-7% in humans and 5-15% in most animals) of escalated aggressive individuals is a major source of death and disability, thus constituting one of the most significant problems for the public health and veterinary medical institutions. Obviously, there is an urgent need to understand these problematic behaviors in terms of their underlying causal mechanisms and modulating factors. In general, animal models are essential to obtain experimental support of the causal nature of physiological and environmental factors in determining particular behavioral expressions. As a matter of fact, a considerable part of our current knowledge on the ethology, etiology, neurobiology, genetics and pharmacology of human aggression is based on experimental and laboratory studies of aggressive behaviors in a wide variety of animals (i.e., ranging from fruit flies, honeybees, ants, crickets, zebra fish, songbirds such as song sparrows and zebra finch, mice, rats, hamsters, prairie voles, dogs, cats and monkeys).

Most neurobehavioral and pharmacological studies of aggressive behavior in the laboratory setting are performed on rodent species (rats, hamsters, voles and mice) that can show high levels of territorial aggression characteristic of their generally dispersive social structure under low population densities in their natural habitats. Therefore, much of the preclinical aggression research is conducted in territorial male resident rats/mice confronting an intruder conspecific. This so-called resident-intruder paradigm allows the spontaneous and natural expression of both offensive aggression and defensive behavior in laboratory rodents in a semi natural laboratory setting. By recording the frequencies, durations, latencies and temporal and sequential patterns of all the observed behavioral acts and postures in the combatants during these confrontations, a

detailed quantitative picture (ethogram) of offensive (resident) and defensive (intruder) aggression is obtained. The resident-intruder paradigm brings this natural form of behavior into the laboratory allowing controlled studies of both the resident aggressor and the intruder victim (Koolhaas et al., 2013). The paradigm is strongly based on the fact that an adult male rat will establish and defend a territory when given sufficient living space, resources and mating partners. Territoriality is significantly enhanced in the presence of females and/or sexual experiences. As a consequence of territoriality, the resident will attack unfamiliar males intruding in its home cage. The intruder in turn will show defensive behavior in response to the offensive attack by the resident. Although typical patterns of aggressive behavior differ between species, there are several concordances in the ethology and neurobiology of aggression among rodents, primates and humans.

Aggressive Behavior: Different Forms in Both Animals and Humans

The existence of different kinds of aggression has long been recognized mainly on the basis of animal research (Blanchard & Blanchard, 1981; Brain, 1979; Adams, 2006). There are generally two types of attacks in both males and females: offensive and defensive. These differ in motor patterns, bite/attack targets, ultimate functional consequences and proximate neurobiological control mechanisms. Basically, **Offensive aggression** can be defined as a form of social communication principally aimed at the (pro)active control of the social environment. The motor patterns for offensive aggression are chase, offensive upright posture, offensive sideways posture, attacks (simple bites or bite and kick), piloerection (bristling) of the fur and teeth-chattering (mainly in rats) or tail-rattling (mostly in mice). In the minutes leading up to intense attack bites, the resident rat emits brief pulses of ultrasonic vocalizations in the 50 kHz ranges that may reflect high excitement The bite targets are primarily the hindquarters of the flanks, back and base of the tail (less-vulnerable body regions). The function is to obtain and retain resources such as space, food, and mates. **Defensive aggression** can be defined as a set of social behaviors performed in defense to an attack by a conspecific or a potential predator. The motor patterns for defensive aggression are flight, defensive upright posture, defensive sideways posture (keep-away), and attacks (lunge and bite). These defensive motor acts are usually accompanied with urination/defecation and emittance of 22 kHz ultrasonic vocalizations. The bite targets are primarily the face (snout), neck, and belly (vulnerable body regions). Defensive aggressive behavior differs from

offensive aggression in that bite attacks are not signaled in advance by threats. The function is to defend one's self, mates, and progeny from attacks of another animal of the same or different species. For example, a dominant resident against an unfamiliar male conspecific intruder of the home territory displays offensive behavior (territorial aggression). The offense-defense distinction plays a prominent role in understanding the biology and physiology of animal aggression.

Different forms of aggression are also recognized in humans and the offensive pattern of aggression in animals generally relates to the “hot-tempered” **hostile aggression** subtype in humans (also called **reactive**, **emotional**, **affective**, and **impulsive** aggression). The most basic acts of aggression in humans are: hitting, kicking, biting, pushing, grabbing, pulling, shoving, beating, twisting and choking. Threatening (vocal) and using objects (weapons) to aggress are also included into this definition (Tremblay, 2010). This form of aggression has its strong initiative engagement and autonomic/neuroendocrine arousal in common with offensive aggression in animals. Moreover, both in animals and humans, this form of aggressive behavior is usually initiated in response to a perceived threat such as the intrusion of an unfamiliar conspecific into the territory or in response to fear and frustration (omission of expected rewards). In contrast, “cold-blooded” **instrumental aggression** (also called **premeditated** and **proactive** aggression) is callous-unemotional aggression that seems to resemble more the quiet-biting attack or predatory forms of aggressive behavior in rodents.

Although both male and female rodents perform offensive aggression, there is a clear gender difference in the frequency and intensity of aggression similar to what is generally observed in humans. Males may perform frequent and fierce offensive aggression in a territorial and socio-sexual context. Females show defensive maternal aggression mostly in a maternal context, but low to medium levels of offensive aggression can certainly be observed in all female groups in relation to competition within the social hierarchy (De Jong et al., 2014).

Finally, it should be noted that aggression in both animals and humans has to be conceptualized into two components: trait-like aggressiveness and state-like aggressive behavior. Whereas **trait-like aggressiveness** refers to an individual’s predisposition to act persistently aggressive in various different contexts, **state-like aggression** refers to the actual execution of aggressive behaviors. This distinction appears to be of crucial

importance when linking certain physiological or neurobiological parameters to aggression.

Development of pathological or deviant forms of resident-intruder aggression.

Until approximately a decade ago, most animal studies of aggression were concerned with the ultimate and proximate mechanisms of normal adaptive aggressive behavior, while clinically the focus was predominantly on violent individuals and excessive or inappropriate forms of human aggression. Besides several political, ethical, funding and translational constraints, the lack of biologically relevant and valid animal models for these pathological forms of aggressive behavior is one important reason for the gap in our knowledge about the neurobiological roots and developmental mechanisms of violence in humans. Therefore, new experimental models in preclinical research are being developed that focus more on provoking escalated and uncontrolled forms of aggressive behavior in order to capture the problematic clinical phenotype. Ideally, such models should demonstrate excessive, injurious and impulsive aggressive behavior that exceeds and/or deviates from normal species-typical levels or patterns (see Miczek et al., 2007; 2013; 2015; de Boer et al., 2009;). However, a major obstacle in preclinical animal aggression research is that most laboratory rodent strains are very placid and docile compared to their wild ancestors. In virtually all commercially available laboratory mouse (>500) and rat (>250) strains today, the aggressive behavioral traits, including the putatively underlying molecular genetic components, are dramatically compromised in terms of absolute level and variation. Most likely, this is the result of (unintended) artificial selection for tame and tractable behavior during the century-long domestication process of this wild-caught animal, being kept, reared and bred in captivity (de Boer et al. 2003)**.** A classic example of this is the maintenance of docile characteristics long after selection for tameness in wild silver foxes even though the behavioral selection criteria are no longer applied, indicating that alleles that predispose to aggression have been removed from the population (Belyaev, 1979)**.**

Consequently, to obtain appreciable levels of offensive aggression in these constitutionally docile laboratory strains, several procedural (often rather artificial) manipulations have been employed to promote and/or enhance the tendency to display offensive aggressive behavior (Table 1 and see de Boer et al. 2009; Natarajan and Caramaschi 2010 for review).

INSERT Table 1 here

Table 1. ***Procedural manipulations to induce heightened and/or escalated aggressive behavior***.

- **Behavioral/environmental procedures**
  - **Prolonged social isolation (adolescence)**
  - **Maternal neglect/early-life stress**
  - Noxious stimulation
  - Social provocations/instigations
  - Frustrative non-reward experiences
  - **Repeated winning (pleasure)-enhanced**
- **Genetic procedures**
  - **Genetic selection/individual predispositions**
  - Genetic manipulations (targeted deletions, overexpression)
- **Pharmacological/neurobiological procedures**
  - **Brain stimulation/lesion enhanced**
  - **Alcohol-heightened**
  - **Adolescent exposure to cocaine or Androgen Anabolic Steroids (AAS)**
  - **Glucocorticoid hypoactivity**

Obviously, and validly so, some of these procedures have been adopted with the intent to mimic the conditions under which violent behavior in humans occurs (e.g., frustration, maltreatment and stress, instigation, alcohol, drug and anabolic steroid use). Although these experimentally heightened forms of aggressive behavior may to some extent resemble more intense forms when compared to their already low species-typical rates of aggression, they may still fall into the normative range when compared to the patterns and levels of their wild ancestors. Indeed, higher levels and wider-ranges of spontaneous intraspecific aggression are encountered in feral (wild-derived) or semi-natural populations of rats and mice as compared to their laboratory-bred conspecifics (de Boer and Koolhaas 2003; figure 1).

INSERT Figure 1 here

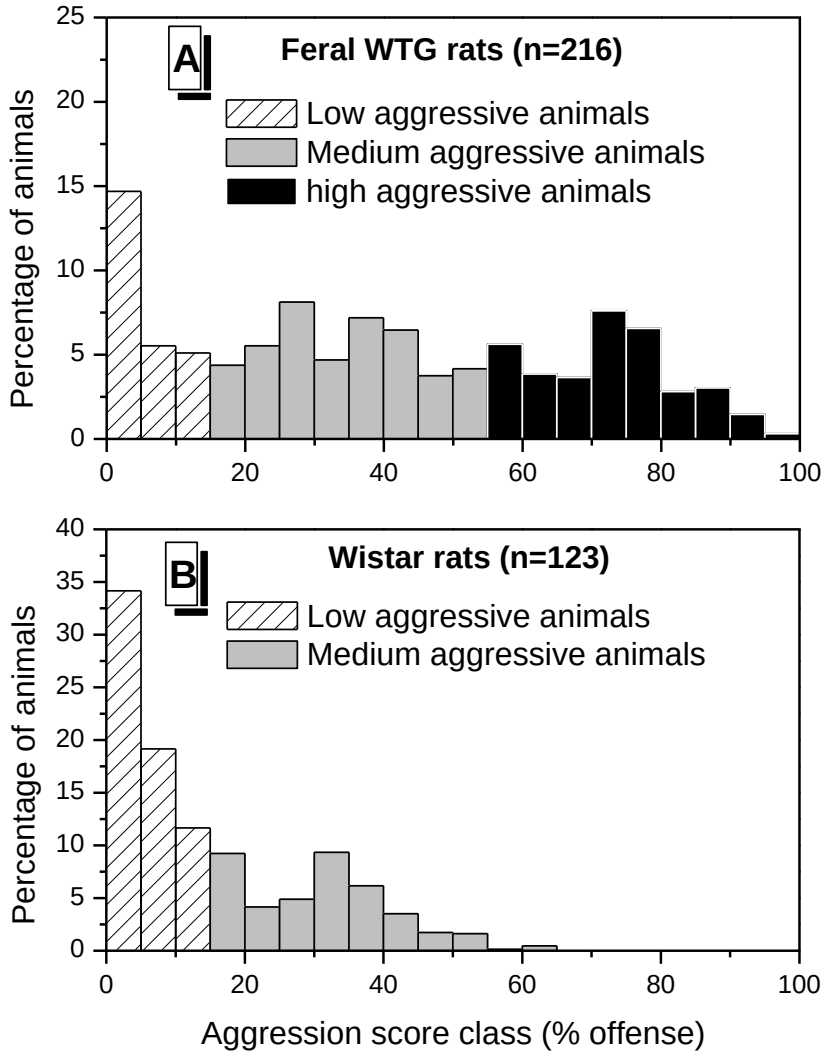


Figure 1. *Frequency distribution of offensive resident-intruder aggression in a population of unselected feral Wild-Type Groningen rats (A) and domesticated laboratory Wistar rats (B). Rats are categorized according to their level of aggressive behavior expressed as percentage of time in the RI-test. Note that the highly (> 55 % aggressive behavior) aggressive phenotype is virtually absent in the domesticated rat strain (de Boer et al. 2003).*

Therefore, an increase in solely the frequency and duration of aggressive acts is only one component of “pathological” aggressive behavior. More productive and relevant animal models of excessive and abnormal forms of aggression should basically

demonstrate intense and/or injurious aggression that exceeds normal species-typical levels and patterns. In other words, a form of aggressive behavior that is not subject to inhibitory control anymore and has lost its function in social communication. Hence, this loss of the social communicative nature of the aggressive interaction in the currently available animal models of escalated aggressive behavior are operationally-defined by: (a) Low provocation threshold, short latency to initiate attack; (b) High rate and intensity, leading to significant tissue damage; (c) Disregard of appeasement signals, (d) Lack of species-normative behavioral structure (i.e., attacks are deficient in conveying signaling intention, and lack of context in that critical features of the opponent such as age, sex or situation are misjudged) (Miczek et al., 2013; 2015; Haller & Kruk, 2006, Nelson & Trainor 2007; De Boer et al., 2009). Several of these signs and symptoms of violent-like aggressive display are reliably engendered in the following animal model that has achieved, at least to a variable extent, similarity with human violent aggression in terms of symptomatology and phenomenology (face validity), phylogenetic and ontogenetic origins (construct validity) and response to clinically established treatments using clearly understood neurobiological mechanisms (predictive validity).

Escalated aggressive behavior in unselected feral animals and selective breeding for escalated aggression.

Feral or semi-natural populations of rats and mice display much higher levels and a broader range of innate and normal adaptive offensive aggression compared to their highly domesticated laboratory-bred conspecifics (see Figure 1). More interestingly however, clear escalated aggressive and violent characteristics, as defined above, can be engendered in approximately 10-15% of these constitutionally medium to high-aggressive rats that experience repeated victorious episodes of social conflict (i.e., by permitting them to physically dominate other conspecifics for more than 10 times) (see Figure 2). Like humans and other animals, most individual rats respond to these repetitive social conflicts with appropriate and well-controlled functional forms of aggressive behavior, while only a small fraction demonstrate escalated aggression and become violent and destructive. Enhanced levels of offensive aggression and an increased probability of winning an aggressive encounter following previous victories (the so-called “trained fighter” or “winner” effect) was originally already described by Ginsburg and Allee in 1942, and since then has been demonstrated frequently in a wide variety of animal species (see Hsu et al., 2005, for review). Similarly, male wild-derived

house mice that were artificially selected for short-attack latencies (i.e., high aggressive SAL mice) show virtually all of the above-mentioned signs of violent aggressive behavior already after 3-5 repeated winning experiences (Natarajan, 2010; Caramaschi et al., 2008).

INSERT Figure 2 here

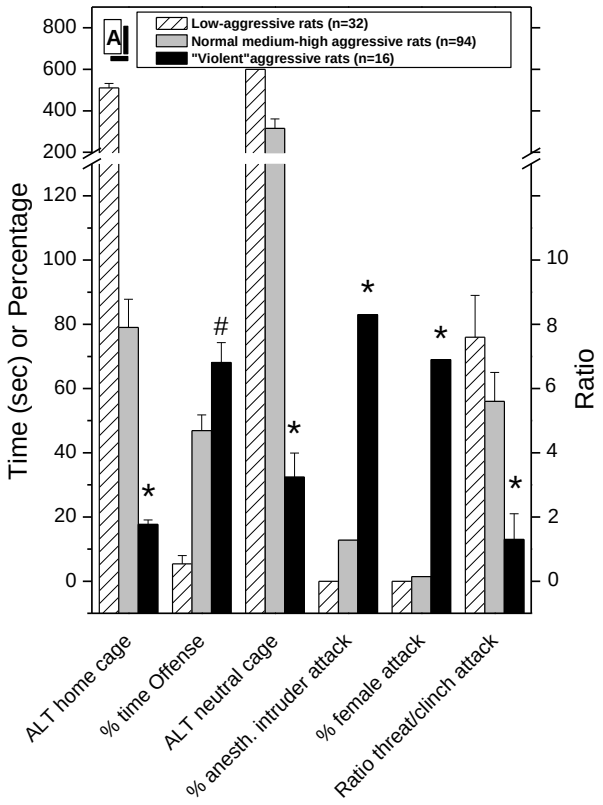


Figure 2. *Normal and 'violent' aggressive behavioral characteristics in a small fraction (10-15 %) of resident wild-derived WTG rats before and after multiple (>10) victorious experiences * indicates significantly ($p<0.05$; student t-test) different from untrained and trained normal aggressive groups.*

Thus, upon positive reinforcing or "pleasurable" victorious social experiences, a small group of constitutionally aggressive rats and artificially selected aggressive mice

are very prone to show a breakdown of the aggressive behavioral inhibition mechanisms, and transform their initial functional adaptive aggressive behavior into a more violent-like and pathological behavior.

Numerous studies in a wide variety of animal species have convincingly demonstrated that in addition to securing access to resources, the most intriguing consequence of winning an aggressive conflict is the self-reinforcing or rewarding effect of this type of behavior. Actually, individuals seek out the opportunity to fight and engage in aggressive behavior as a source of pleasure. The most convincing evidence that aggression is rewarding to animals is that the opportunity to engage in aggressive behavior can reinforce operant responding, i.e., animals are willing to work (e.g., bar pressing, nose poking) for aggression as a source of reward and satisfaction (see Miczek et al., 2004 for review). Not surprisingly, just like other positive reinforcers such as food, drugs or sex, the mesocorticolimbic dopamine system is associated with the incentive salience of the rewarding properties of winning fights: Nucleus accumbens (NAcc) dopamine is strongly released during (anticipation of) aggressive episodes (Ferrari et al., 2003) and pharmacological antagonism of dopamine D_1/D_2 receptors in the NAcc diminishes the seeking of the opportunity to fight (Couppis & Kennedy, 2008). In addition, direct optogenetic activation of ventral tegmental area (VTA) dopamine neurons increases aggression (Yu et al., 2014), proving that dopamine function and aggression are causally linked.

This animal model translates well to impulsive aggressive and violent behavior in humans, and in particularly affords the opportunity to identify the plastic neuromolecular changes in the “aggression” control systems that are hypothesized to underlie a shift of normal adaptive aggression into more violent forms (Sluyter et al., 2003; de Boer et al., 2009). For example, in mice that have won territorial disputes repeatedly, a selective enhancement of androgen sensitivity in neural pathways related to motivation (VTS and NAcc) and social aggression (BNST) was observed (Fuxjager et al., 2010). In addition, profound functional changes in the key regulatory sites (5-$HT_{1A/B}$ autoreceptors and reuptake transporters) that control the (re)activity of serotonergic neurons were found to be causally related to the transition into excessive forms of aggression (de Boer et al., 2015).

Neurobiological Correlates of Aggression and Violence

Research of the neurobiology of aggression started with a classic approach of surgical lesioning or electrically stimulating specific brain areas. These experiments were initiated more than 100 years ago with the taming effects of temporal lobectomies in rhesus monkey by Brown and Schafer (1888) and extremely aggressive dogs by Friederich Golz (1890) followed approximately thirty years later by Philip Bard's (1928) demonstration that rage-like aggressive behaviors were absent in posterior hypothalamic knife-cut transectioned cats. Around that same time period, Walter R. Hess started his Nobel Prize-winning intracranial stimulation (ICS) experiments in cats, demonstrating aggressive responses evoked by electrical stimulation of the hypothalamic brain region (Hess). Since then, neuroscientists have sought to understand the neural basis of aggression and violence by perturbing and monitoring brain activity through a variety of methods and in a wide variety of animals such as monkeys, dogs, cats, rats, mice, voles and hamsters. By employing numerous increasingly sophisticated tools of functional neuroanatomy (i.e., from the classic electric/chemical lesion and stimulation techniques to neurochemical mapping and manipulations), many important strides have been made in understanding the functional brain circuit organization of different social (aggression, sex, parental care) behaviors, i.e., the structurally and functionally highly interconnected "social behavior neural network" (SBN) (Newman, 1999; Nelson & Trainor, 2007).

A Highly Interconnected Network of Brain Regions Controls Aggression

To more comprehensively identify this SBN, and particularly the specific neural circuitry involved in aggressive behaviors, determining the pattern of activation of immediate early gene expression has been employed successfully within the last two decades. Fos is the protein product of an immediate early gene (IEG), *c-fos*, which is expressed in neurons shortly after their depolarization (activation), and then induces expression of downstream genes. Fos-expression can be visualized using immuno-histochemical staining techniques and the number of Fos-positive neurons in each brain area is used to quantify the activation the area. Application of this technique in offensive aggression paradigms in rats, mice, and hamsters reveals a neuronal network that includes (but is not limited to) the intimately interconnected forebrain (limbic) structures like cortico-medial amygdala (MeA and CoA), bed nucleus of the stria terminalis (BNST), lateral septal area (LS), mediodorsal and anterior thalamus, several hypothalamic nuclei including the anterior hypothalamus (AHA), ventromedial hypothalamus (VMH), lateral

hypothalamus (LH), the paraventriculaire nucleus (PVN), the medial prefrontal cortex (mPFC), the midbrain periaqueductal gray (PAG), dorsal raphe nucleus (DRN), locus coeruleus (LC) and ventral tegmental area (VTA) (see Figure 3 and de Boer et al., 2015 for a more detailed review on the neurobiology of offensive aggression). Comparative research indicates that this highly interconnected neuronal network for offensive aggression is remarkably similar in many vertebrate species including humans, indicating that it is evolutionary ancient and very well conserved (Goodson, 2005; O'Connell & Hofmann, 2012). Indeed, this interconnected brain aggression circuitry is generally confirmed in humans by modern brain-imaging techniques such as Functional Magnetic Resonance Imaging (fMRI) and *Positron Emission Tomography* (PET) that allow the *in-vivo* analysis of entire neuronal networks involved in certain types of aggressive behavior. However, it is quite surprising that in most of the human neuroimaging studies, the hypothalamic limbic brain structures involved in the direct control of animal fighting and attack usually do not show up in their region of interest analyses. Rather, these studies predominantly focus on the higher cortical (i.e., prefrontal, cingulate) and temporal lobe (amygdala) brain structures.

The function of the "SBN" brain areas in the expression and control of aggressive behavior ranges from sensory processing and perception up to the generation of somatomotor output patterns, the autonomic and neuroendocrine support of behavior, and all organizational processes in between. However, although the neural circuitry of aggressive social behavior is mapped relatively well in terms of brain (sub)nuclei and its interconnections, it still remains a challenging task to decipher how the activity of distinct sets of neurons within the various nodes of this basic SBN circuitry give rise to different phases (initiation, execution and termination), levels and/or forms of aggressive behavior.

The Hypothalamus as a Critical Brain Region for Offensive Attack

Of all the areas in the brain, the hypothalamus is by far the best-studied region in relation to aggression, ever since seminal lesion experiments found suppression of raging aggressive acts in cats (Bard, 1928) and intracranial stimulation experiments induced this behavior (Hess, 1943). With the development of appropriate stereotaxic instruments, an extensive series of groundbreaking lesion- and electric stimulation studies defined the attack area in the hypothalamus, hence called the hypothalamic attack area (HAA). This HAA consist of an area extending between the LH and the VMH

rostrally alongside the anterior hypothalamic nucleus (see Kruk, 2014 for detailed review). Electrical stimulation of parts of the HAA has been reported to induce fierce attack behavior in a variety of animals (e.g., rats, cats, monkeys). This hypothalamically-induced attack behavior can be directed against males, females, anesthetized, or even dead rats, and is directed toward vulnerable body parts. Hence, this form of induced aggression is clearly abnormal and violent-like.

However, despite its anatomical precision, electrodes still affect a rather ill-defined population of neurons and fibers of passage that do not allow definite conclusions on the precise neuronal and circuit-level mechanisms underlying offensive attack. The brain packs roughly 100,000 neurons and a billion synaptic connections in every cubic millimeter of tissue, and electrically stimulating or lesioning even a tiny location in the brain will excite/silence a very large number of intermeshed cells of different kinds. Recently, newly emerging techniques for mapping, measuring, and manipulating neural activity based on genetic targeting of specific neuron subtypes has solved many of these problems. In particular, optogenetics and pharmacogenetics have recently made it possible to rapidly and reversibly activate or inhibit small molecularly distinct populations of neurons (anatomical and genetic precision) at any moment in time (temporal precision) (Anderson, 2012; Deisseroth, 2014). These revolutionary techniques offer the ability to selectively manipulate individual neural circuit elements that underlie aggression-relevant behaviors. The first experiments investigating the role of the hypothalamus in the regulation of aggression using optogenetic stimulation focused on the ventrolateral subdivision of the VMH. Following virally-delivered expression of the light-sensitive protein channelrhodopsin-2 (ChR2) in this VMHvl region of mice, light pulses delivered through an implanted optic fiber produced robust offensive attacks directed toward male mice, castrated male mice, female mice, and inanimate objects (Lin et al., 2011). Accordingly, inhibiting these neurons using virally-expressed *C elegans* ivermectin-gated chloride channel, which prevents the initiation of action potentials by hyperpolarizing the neurons upon ligand binding (i.e., a pharmacogenetic approach), suppressed normal attacks. Subsequent studies have capitalized on the fact that the neurons of the VMHvl are primarily glutaminergic and are enriched with estrogen receptors of the alpha subtype (Er_{α}). Both Er_{α}-knockout mice and RNAi knockdown of Er_{α} in the VMHvl resulted in a dramatic decrease of natural inter-male aggression (Sano et al., 2013). Most recently, optogenetic stimulation of Er_{α} VMHvl neurons triggered attack behavior whereas optogenetic inhibition suppressed fighting, suggesting that Er_{α}

neurons in this small hypothalamic area are necessary and sufficient to initiate and terminate bouts of aggression (Lee et al., 2014). Beside Er_{α}, neurons in the VMHvl also express a variety of other neuromodulator receptors, including serotonin 1A, 2A, 2C, muscarinic acetylcholinergic, and oxytocin receptors. Since many neuromodulators such as serotonin, dopamine and oxytocin change their levels dynamically during the course of aggressive behaviors, they may influence VMHvl neuron excitability and hence aggressive attack. Similar type of opto/pharmacogenetic interrogations and viral vector-based approaches in rodent models of aggression are recently being performed in various other nodes of the brain social aggression circuitry, i.e., amygdala (Hong et al., 2014), prefrontal cortex (Takahashi et al., 2014) and VTA (Yu et al., 2014) and illuminate the precise neuromolecular determinants of aggressive behavior in both its normal and excessive forms.

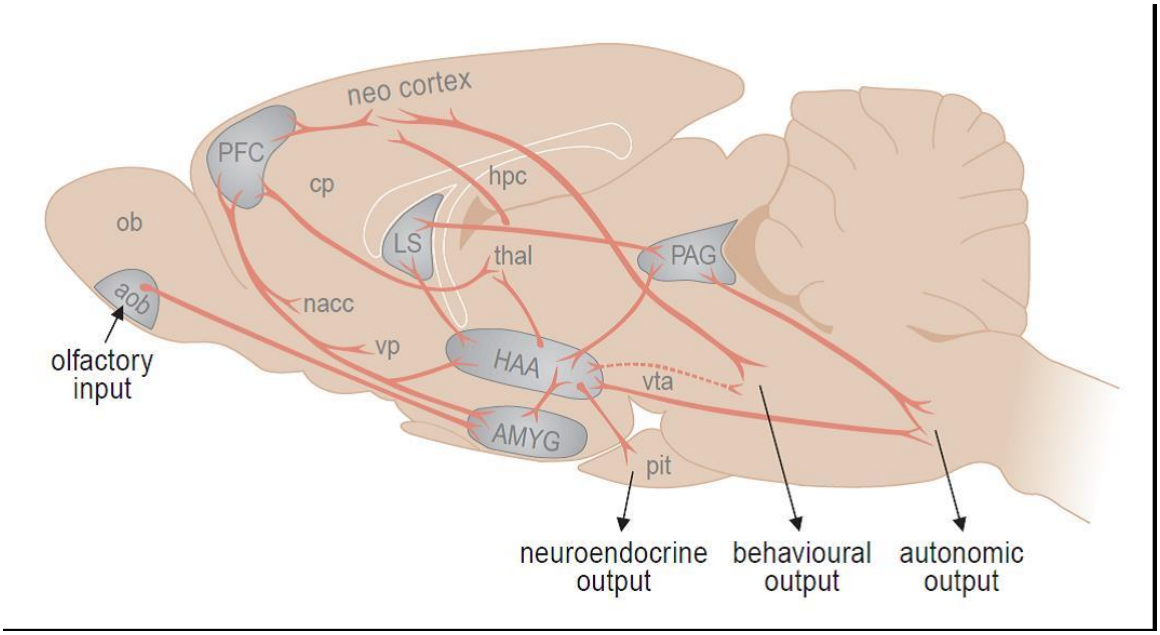


Figure 3: *A scheme of the neuronal network in rodents involved in aggressive behavior and the organization of the accompanying neuroendocrine and autonomic activation. (aob=Accessory olfactory bulb; AMYG= amygdala; cp= caudate putamen; hpc = hypothalamus; hpc= hippocampus; LS= lateral septum; nacc= nucleus accumbens; ob= olfactory bulb; PAG= Periaqueductal gray; PFC= Prefrontal cortex; pit= pituitary; thal= thalamus; vp= ventral pallidum; vta= ventral tegmental area).*

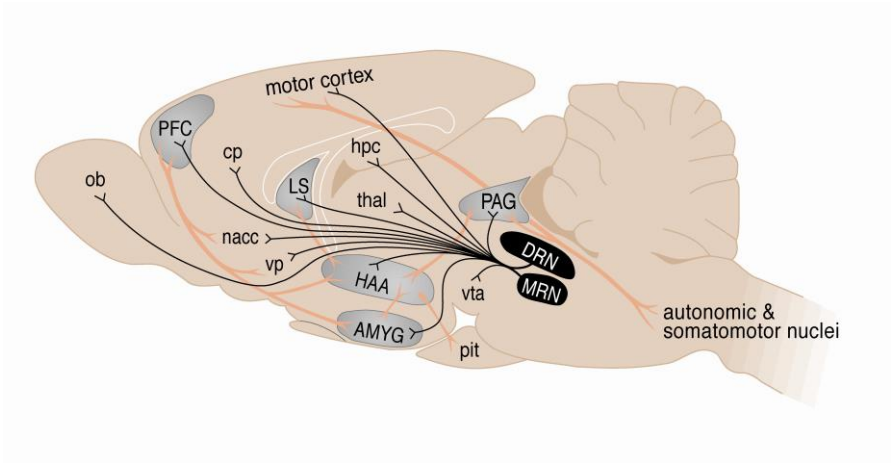
Neurochemical Modulation of the Aggressive Neural Network

Obviously, the functional activity of this entire social behavior neural network, and thereby the selection of the appropriate behavioral response to social challenges and opportunities, is determined by a wide variety of molecular substrates (i.e., neurotransmitters, hormones, cytokines, and their respective metabolic enzymes, receptors, and intraneuronal signaling molecules). Undisputedly, among the neurochemical systems that are considered key signaling molecules in this neurocircuitry controlling aggression are the monoamines serotonine (5-HT) and dopamine (DA), the 'social' neuropeptides oxytocin (OXT), and vasopressin (AVP), the 'stress' neuropeptide corticotropin releasing factor (CRF), the 'stress' HPA- and 'sex' HPG-axis's steroid hormones (corticosterone, testosterone, estrogen), and their cognate receptors. Indeed, several studies in wild-type rats and artificially-selected SAL and LAL mice show a widespread central nervous differentiation between the high and low aggressive extremes, for example at the level of the oxytocinergic modulation of the central nucleus of the amygdala (Calcagnoli et al., 2015), the vasopressinergic neurons in the bed nucleus of the stria terminalis and its innervation (density) of the lateral septum (de Boer et al., 2015), the auto-inhibitory control mechanisms of serotonin neurotransmission (see next section). However, the exact functional role of these neurobiological systems in the generation of a particular behavior and/or their behavioral specificity is still far from clear. Moreover, with the notable exception of serotonin signaling components, the causal involvement of these neurobiological substrates in determining aggressive behavior requires further experimental evidence employing the novel opto- and pharmaco-genetic manipulation techniques.

Serotonin is the main molecular orchestrator.

All nodes in the neuronal network for offensive aggression are substantially innervated by serotonergic (5-HT) neurons originating in the dorsal and median raphé nuclei in the brain stem (see Figure 4). More than any other neurochemical system, this evolutionary ancient and extremely well conserved neurotransmitter system is generally considered the primary molecular orchestrator of aggressive behavioral traits in virtually every animal species, including man (Siever, 2008; Nelson & Trainor, 2007). However, the direction and exact causal linkage of this association is very complex and it has proven notoriously difficult to unravel the precise role of this amine (and every facet of its synthetic and metabolic pathways, uptake and storage processes, and dynamic receptor signaling mechanisms) in the predisposition for and execution of aggressive behavior in

both its normal and pathological forms. For decades, high levels of aggressive behavior are believed to be associated with low brain 5-HT neurotransmission activity. This frequently reiterated serotonin deficiency hypothesis seems consistent with the fact that serotonergic receptor agonists used to mimic higher serotonergic activity, generally reduce aggressive behavior. However, recent studies of the functional status of the 5-HT system before, during, and after the execution of normal adaptive and abnormal pathological forms of aggression have led to a somewhat different view. Display of normal adaptive offensive aggressive behavior aimed at territorial control and social dominance is associated with a higher 5-HT neuronal activity (see de Boer et al., 2015 for relevant references). A negative correlation between aggression and 5-HT as captured in the deficiency hypothesis seems to be a trait-like characteristic of pathological forms of aggression (e.g., violence). For example, a clear positive correlation was found between the level of normal adaptive expressions of offensive aggression and basal cerebrospinal fluid (csf) concentrations of 5-HT and/or its metabolite 5-HIAA. A significant negative correlation between aggression and 5-HT levels was found only upon inclusion of samples from abnormally- and excessively aggressive trained fighter animals. A critical evaluation of the csf 5-HIAA data in aggressive humans confirms this idea that the serotonergic deficiency appears to hold in particular for specific groups of individuals who persistently engage in more aberrant, impulsive and violent forms of aggressive behavior rather than in individuals with instrumental (functional) forms of offensive aggression.



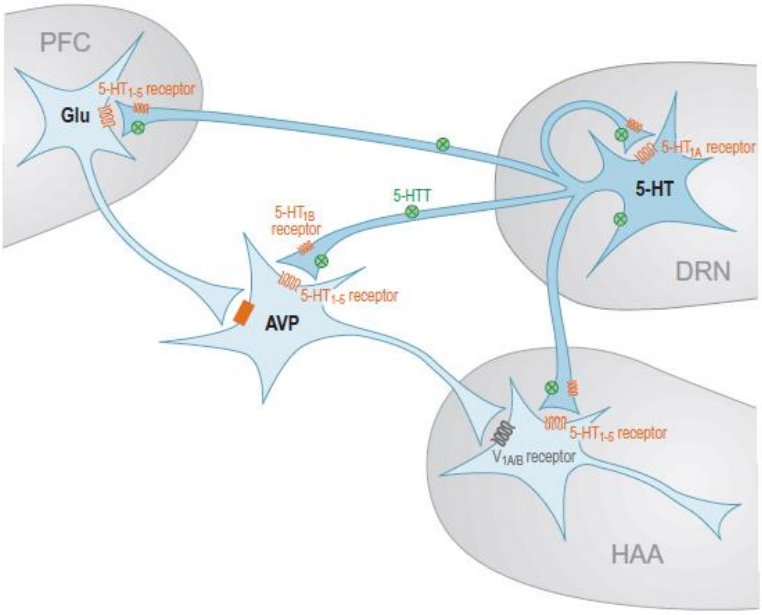


Figure 4: *Serotonergic control of the social behavior neuronal network. See legend of figure 3. DRN = dorsal raphé nucleus; MRN = medial raphé nucleus*
4B: More detailed neuromolecular characteristics of part of the DRN-prefrontal-HAA microcircuitry involved in the control of aggressive behavior. Glu = glutamate; AVP = arginine vasopressin; 5-HT = serotonin; 5-HTT= serotonin transporter.

Treatment with 5-HT_{1A} or 5-HT_{1B} receptor agonists is one of the most potent pharmacological methods to selectively suppress aggressive behavior in a variety of animal species and experimental paradigms. Apart from acting on receptors at postsynaptic sites, these two receptor agonists also affect the two main serotonergic auto-receptors involved in the negative feedback control of the 5-HT neuron at the level of the synapse (5-HT_{1B}) and at the level of the cell soma (5-HT_{1A}) (see Figure 4). Hence, activation of these receptors by agonists will potently activate the negative feedback and thereby reduce 5-HT firing and neurotransmission. It appears that the potent anti-aggressive effects of these compounds are largely expressed via their action on these inhibitory auto-receptors located at the cell soma and the nerve terminal, by attenuating intruder-activated 5-HT neurotransmission (de Boer et al., 2015).

Interestingly, highly aggressive animals are characterized by upregulated somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptor functionality. This considerably (approximately 20-fold) enhanced tonic inhibitory control of serotonergic neurons in

aggressive males may explain the negative correlation between baseline levels of 5-HT and escalated aggression found in many species. Furthermore to signify the causality of this correlation, 5-HT_{1A} autoreceptor sensitivity increased or decreased upon enhancing (by repeated victorious experiences) or attenuating (by repeated defeat experiences) aggressiveness, respectively. Notably, animals that escalated their aggressiveness and started to engage in violent-like aggressive behavior demonstrated 5-HT_{1A} autoreceptor super-sensitivity. More persuasively, recent molecular genetic studies have shown that transgenic mice with conditional (at adult age) overexpression of somatodendritic 5-HT_{1A} autoceptors demonstrate suppressed 5-HT neural firing that was associated with a profound hyper aggressive behavioral phenotype (Audero et al., 2013). These data confirm the causal role of tonic 5-HT activity in setting a trait-like threshold for executing overt aggressive behavior.

Vasopressin and Oxytocin as important neuropeptidergic modulators of the social behavior network.

Besides their important peripheral physiological functions as neurohypophysial-released hormones, the neuropeptides arginine vasopressin (AVP) and oxytocin (OXT) are also implicated in inter-neuronal communication within various nodes of the social brain network to modulate emotional and social behavioral and physiological responding (Lee et al., 2009a). AVP is generally known to increase anxiety-like behaviors, stress and aggressiveness, whereas OXT has the opposite effects and facilitates social attachment, care, and affiliation (Heinrichs et al., 2009). Existing data from early pioneering work on these neuropeptides convincingly demonstrated opposite roles for AVP and OXT in fear learning processes (Bohus & de Wied, 1998). More recent studies in our wild-type rats and/or artificially selected aggressive (SAL) and non-aggressive (LAL) house mice have demonstrated that high-aggressive animals exhibit higher levels of AVP release when compared to their non-aggressive counterparts (Koolhaas et al., 2010). In addition, there is abundant experimental evidence to support a causal function of vasopressin in proactive aggressive behavior and OXT in passive affiliative behavior. Direct micro-infusion of AVP or OXT into the cerebral ventricles or in selected brain regions facilitates or suppresses, respectively, offensive aggression (Calcagnoli et al., 2015). In addition, a positive correlation between levels of CSF vasopressin and life history of general aggression as well as aggression towards individuals (Lee et al., 2009) has been reported, whereas impaired brain OXT-ergic signaling has been implicated in

several human neuropsychiatric disorders associated with social deficits, impulsivity, and excessive aggression (Lee et al., 2009b). Furthermore, mutant mice with the vasopressin receptor V1A/B gene deleted showed virtually no offensive aggressive behavior anymore, whereas elevated aggressiveness was found in mice with deletions of the OXT receptor gene. Consistent with the aggression-promoting of brain AVP, systemic as well as intra-hypothalamic administration of AVP V1A/B receptor antagonists effectively block offensive aggressive behavior in male hamsters and WTG rats (Blanchard et al., 2007; Koolhaas et al., 2009). Basically, an opposite picture seems to emerge for brain OXT signaling. Recent ethopharmacological studies have clearly demonstrated that enhancement of brain OXTergic function, using both intraventricular, intra-amygdalar, and even intranasal administration routes, produced marked anti-aggressive and pro-social affiliative effects that are dose-dependent, behavior- and receptor-selective, and long-lasting (Calcagnoli et al., 2013; 2015).

Based on the findings outlined above, it can be hypothesized that an endogenous balance between vasopressin and oxytocin signaling within (components of) the social behavioral neural circuit may gate the expression of either aggressive or affiliative responses to salient social stimuli.

Synthesis and outlook

A large body of animal neurobehavioral research convincingly demonstrates that abnormal expressions of aggressive behavior principally find its origin in a dysregulation of the deeply rooted neuronal circuits and/or neurochemical pathways in the brain that mediate normal social affiliative-aggressive behaviors. This highly conserved neural and gene expression brain network encompasses neurons in the mesencephalon projecting to hypothalamic nuclei, amygdaloid, septal, prefrontal, and hippocampal forebrain regions, striatal and thalamic loops with the frontal and prefrontal cortex, as well as important feedback loops to limbic and mesencephalic nuclei. The structural and functional properties of this social behavior brain network are established and constantly shaped by a dynamic interplay of genetic and environmental factors (stress, maltreatment, vicarious experiences, substance abuse) in particular during certain sensitive (i.e., perinatal and adolescent) developmental periods. Undisputedly, among the neurochemical systems that are considered key signaling molecules in this neurocircuitry controlling aggression are the canonical monoamines serotonin (5-HT)

and dopamine (DA), the ‘social’ neuropeptides oxytocin (OXT) and vasopressin (AVP), the ‘stress’ neuropeptide CRF, the ‘stress’ HPA- and ‘sex’ HPG-axis’s steroid hormones (corticosterone, testosterone, estrogen) and their receptors. Evidently, recent genetic studies in both human and animals have demonstrated that polymorphisms or mutations in a number of genes regulating the functional activity of these important signaling molecules may confer risk factors, either alone but usually in coaction with (early) stressful life conditions, for development of antisocial aggressive traits. Particularly, from the viewpoint of targeting novel molecular sites for intervention, the intrinsic 5-HT autoregulatory mechanisms (i.e., the presynaptic 5-$HT_{1A/B}$ autoreceptors and 5-HTT), and extrinsic neuropeptidergic (i.e., OXT, AVP and CRF) and steroid receptor (i.e., MR and AR) modulatory influences of 5-HT signaling are emerging as important molecular determinants of escalated aggression regulation. Although early efforts during the 1950s and 1960s to translate preclinical neurobiological aggression research findings into clinical use have a sordid history, the current emerging circuit-level knowledge of the neuromolecular underpinnings of escalated aggression has great potential to guide the rational development of effective therapeutic interventions for pathological social and aggressive behavior in both animals and humans.

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