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Neurobiology of Supporting Syntactic Chains of Self-Grooming in Rodents and Its Biochecmical Characteristics

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Abstract

Grooming is an innate behavior that serves multiple purposes and has a dual nature, reflecting both comfort and stress. Auto-grooming, in particular, is highly sensitive to stressors and can be influenced by natural and synthetic anxiolytics. Researchers believe that rodent grooming can be a valuable tool in translational neurobiological studies, specifically focusing on aberrant grooming, such as the syntactic chain of grooming, which can serve as an experimental model for certain human psycho-nervous disorders.

Keywords

Grooming, Microstructure, Translational Medicine, Clinical Medicine, Stress

1. Introduction

Grooming, also referred to as self-care behavior, is a trait that is present in almost all vertebrates, including humans, and serves to regulate various biological functions [1] [2]. For example, grooming behavior can manifest as a response to stressful situations, thereby mitigating the impact of stressors on the body. Consequently, when external or internal stressors disrupt the typical grooming pattern, it can be employed as an experimental model to investigate behavioral pathology. Recently, a model of grooming pathological cycles in rats has been linked with expression for various central nervous system (CNS) disorders. The analysis of grooming behavior is of utmost importance in neurobiological studies as it provides valuable insights into the underlying functioning of movement coordination, sensorimotor functions, and neuroendocrine disorders. Conse-

quently, grooming research holds significant potential in the field of translational medicine [3] [4].

Grooming abnormalities that occur in various neurocognitive or behavioral pathologies include disruption in both the number and cycle of grooming.

In terms of translational medicine, rodent grooming may, with more or less adequacy and probability, be a model for studying the effects of various modalities of irritants (physical, chemical, biological) on the body, as well as the effects of drugs [5]. The study of grooming mechanisms may help us to study the pathogenesis of some diseases of the CNS (Tourette's syndrome, obsessive-compulsive disorder, Parkinson's disease, schizophrenia, epilepsy, etc.), which are based on basal ganglia and existing pathological changes in the brain [6] [7] [8].

His study delves into the biological aspects of grooming pathologies, specifically focusing on the biochemical underpinnings and alterations that arise in various pathological conditions within the organism. These alterations in grooming behavior manifest when disruptions occur in the biological processes of the body.

The review provided herein centers around an animal model, with findings that hold relevance for extrapolation to any mammalian species. However, it is imperative to consider the inherent species-specific grooming characteristics when applying these results.

2. Grooming Functions

Rodents are known to engage in grooming activities, which can account for 30% to 50% of their vigilant periods. Grooming serves numerous functions, including fur care, thermoregulation, and the distribution of chemicals on the body surface [9]. In addition to its biological functions, grooming is an essential component of behavior and consists of distinctive rituals with a specific sequence [10] [11] [12].

Grooming can manifest in both comfortable and stressful states, highlighting its dual nature. Traditionally, grooming is categorized as a comfortable, stereotypical behavior, along with activities like bathing, yawning, and stretching [13].

However, it can also occur during times of stress, serving as a marker of stressful behavior [4] [14]. The bimodal nature of grooming can be further understood through the lens of zoology [15] [16].

During comfortable states, rodent grooming involves a complex series of genetically determined, stereotypical, and strictly sequential motor actions that are characteristic of the species. It is an evolutionary phenomenon with ancient origins.

In times of stress and discomfort, grooming may take on a "distorted" form, often observed as freezing or other alterations in olfactory movements. This indicates the potential diversity and adaptability of grooming as a behavioral indicator in ethological studies, revealing its association with other characteristics of stress-sensitive behavior [4] [10] [17] [18].

Additionally, animal grooming is influenced by stress hormones and encompasses both behavioral and endocrine components. Grooming increases the secretion of endorphins, which subsequently reduces the levels of alarm-stage hormones in the body. Auto-grooming, in particular, is a behavior triggered by the activation of the hypothalamic-pituitary axis and the release of relevant hormones, including hypothalamic corticotrophin-releasing hormone (CRH) and pituitary hormones such as adrenocorticotropic hormone, vasopressin, and oxytocin. Grooming behavior is enhanced by systemic administration of these hormones, as well as by their administration directly into the ventricles or hypothalamus of the brain.

Therefore, grooming represents a process in which behavioral and endocrine components of stress intertwine. At the same time, grooming increases the secretion of endorphins, which leads to a state of euphoria, reduces anxiety levels, and weakens stress reactions [18].

3. Normal Grooming Stages

The grooming microstructure is a fixed action, a sequence of instinctive behaviors characteristic of a species that has a strictly defined pattern, with their individual elements and time-scattered sequence and complex, which is called sequential organization (patronage).

In rodents, grooming begins with the animal licking its front paws, and then using its two wet paws to clean the nose area with ellipsoidal movements. After completing the nasal cleansing, it uses one paw to clean the vibrissae and eye area. Then it uses both front paws to finish its grooming around its head, ears, and earlobes. The rodent then takes care of the body, licking the abdomen and spine. The last step in consistent grooming is to care for the genitals and tail [13]. This is called cephalous-caudal grooming or bout. Thus, cephalous-caudal progression is a common rule of grooming behavior in rodents that begins in the nose and continues in the direction of the head, body, genitals, and tail [2] [19].

There is a separate syntactic chain in this complex (**Table 1**; **Figure 1**), which consists of four consecutive phases [3] [14] [20].

Table 1. Grooming stages: Pattern composition.

Stage	Stage name	Behavior	Duration (sec)	Count per side/per stage
I	Elliptical stroke	Rostral grooming: licking of the front paws or strokes around the nose and mouth	1	4
II	Unilateral stroke	facial grooming, unilateral nose grooming	0.25	2
III	Bilateral stroke	Bilateral grooming: strokes of the front toes around the ears on both sides, head grooming	0.75	4
IV	Body grooming	The latter moves into other grooming movements (hind paws, genitals, tail scratching, and licking) that do not belong to the syntactic chain	2	1

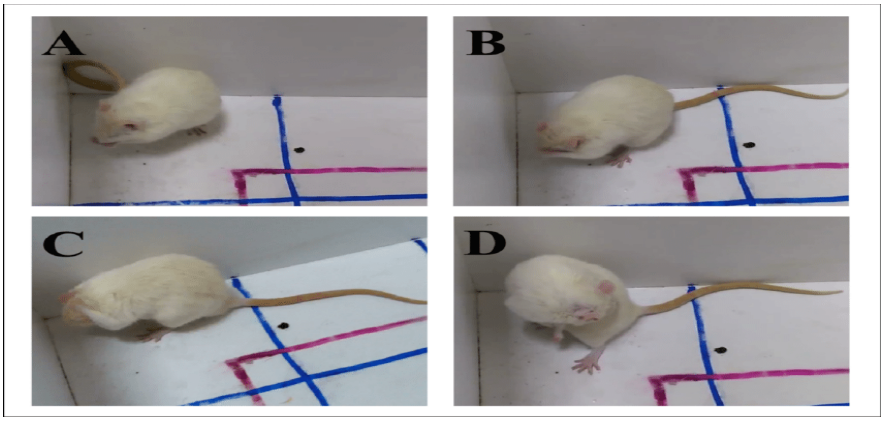

Figure 1. Syntactic grooming chain pattern in rodents: (A) Elliptical stroke (B) Unilateral stroke (C) Bilateral stroke (D) Body grooming.

- Elliptical stroke
- Unilateral stroke
- Bilateral
- Body grooming

During the experimental research, it is methodically convenient to create graphic ethograms of grooming, which perfectly reflect the stages of grooming, their sequence, symmetry, duration, and frequency.

4. Pathological Grooming in Rodents

The study of grooming pathology in rodents encompasses multiple facets, specifically variations in quantity. These variations include instances of absence or, conversely, excessive frequency, persisting for either prolonged or abridged durations. Additionally, disruptions in the sequence of grooming phases, the failure of any phase, irregularities in cycle repetition, such as heightened cycle frequency, abnormal or excessive grooming, the emergence of asymmetry, and the introduction of novel grooming elements, are all considered within the purview of this study.

Of particular note is the concept of "asymmetry" in grooming, which serves as a constructive indicator of normal grooming behavior and, conversely, as a sign of stress.

Overall, abnormal grooming is characterized by high levels of asymmetry (predominantly grooming of one-half of the body), especially during body grooming, although asymmetry is not a necessary indicator of pathology. In mice and rats, for example, grooming differs ethologically in symmetry. Mice have more symmetrical grooming movements than rats [4]. Thus, the asymmetry of grooming as a marker of pathology is more interesting in the case of body grooming [21] [22].

New grooming sequences: An important feature of grooming is the probability of its new grooming sequences, i.e. the initiation of new grooming models. The study of grooming initiations may shed more light on the dynamics and quality of grooming. An important feature of it is the ability to complete the ini-

tiated grooming while maintaining all the sequential stages. The ritual is considered complete if it has reached the grooming of the body and has not stopped during the syntactic grooming, and the genitals and tail - during the cephalous-caudal grooming [23] [24].

Warm-up effect: Initiation/completion of the grooming analysis revealed some other etiological features. In particular, an etiological predictor has been found that the initiated grooming is complete if it achieves body grooming. As it turned out, the speed of ellipsoidal movements around the nose in the initial stage leads to the completion of the grooming ritual. Grooming, completed, begins with faster and more frequent ellipsoidal movements in the nasal area compared to abortion. This phenomenon is called the warm-up effect [4]. The percentage of incorrect transitions of grooming phases (out of the total number) and percentage of interrupted grooming acts (out of the total number of bouts) are used as indicators of stress behavioral parameters and are the only parameters of auto-grooming, the change of which reliably reflects the indicators of stressors in animals [16] [23] [25] [26].

5. Grooming Analysis

There are two methods of grooming analysis: the grooming analysis **algorithm** and the grooming **syntax**.

The first method involves observing grooming globally: including the syntactic chain, as well as stroking and licking of the tail and genitals, and assessing cephalous-caudal expression (from where grooming began - from vibrissae to tail and genitals or messy), as well as the sequence of grooming phases [16] [17].

The second method of grooming analysis involves the **study of grooming syntax**. Behavior according to this method is divided into two parts: chain (syntactic) and non-chain grooming. Chain grooming is involved in a complex of other non-chain grooming movements and other grooming movements that do not belong to syntactic grooming.

6. Neurobiological Basis of Grooming

Neurobiological studies have established that grooming in adult rodents consists of specific and rather stereotypical patterns called syntactic chains, including rostral grooming and body grooming (ending with body grooming). The stereotype, in this case, refers to repetitive behavior (abnormal or excessive), i.e. the performance of the same behavioral movements over a period of time. Grooming syntactic chains contain similar signs to other fixed action patterns, such as sexual and aggressive behavior.

A typical syntactic chain is often involved in other rodent grooming behaviors but differs from it in cephalous-caudal direction (head-to-body) and stereotyping. It accounts for 10% - 15% of grooming behavior [21] [27] [28].

Figure 2 shows different parts of the brain, giving us a simple idea of the parts of the rodent brain that are involved in grooming.

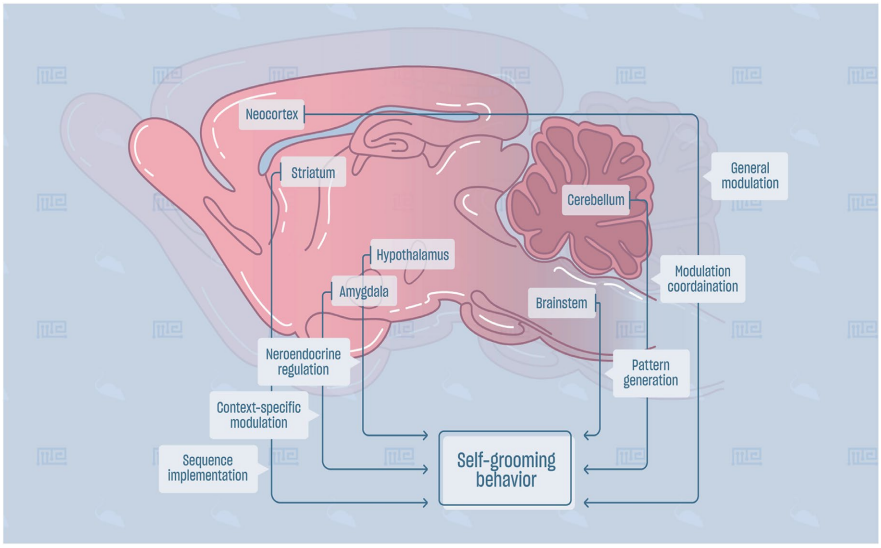

Figure 2. Regulation of rodent self-grooming and the associated brain regions. (Kalueff A.V. et al., 2016).

The **nigrostriatal pathway** and **basal nuclei** of the brain, as well as the effects of various dopaminergic drugs, genetic mutations, and psychogenic stressors, are responsible for sequencing grooming movements and initiating and completing them. The syntactic chain usually intersects with less stereotypical, non-syntactic grooming, which includes stroking and licking of the tail, and genital grooming, the latter accounts for 85% - 90% of all grooming behaviors [16]. Grooming behavior is supported by ethological analysis; it includes both chain and non-chain grooming and is widely used in neurobiological studies [25].

In humans and other mammals, chemically specialized macroscopic zones called striosomes, or "**striatal bodies**," form a distributed labyrinthine system within a large volume of the striatum, which constitutes the extrastriosomal matrix. This architectural arrangement, known as the striosomal matrix architecture, controls the distribution of nearly all neurotransmitters and their receptors, as well as the relative distribution of projection and interneurons in the striatum [29]. Studies have revealed that following dopaminergic stress, striosomes exhibit strong activation and express early response genes encoding transcription factors. This heightened activation of striosomes strongly correlates with increased repetitive behaviors, including self-grooming, in both nonhuman primates and rodents [26] [30] [31] [32] [33].

Brain structures, such as the striatum, substantia nigra, pale nucleus, subthalamic nuclei and others play a crucial role in sequencing behavior [12]. **Basal nuclei**, particularly the striatum and its dopamine input pathways, control the motor behavior of rodents and their sequence. The **neocortex** participates in the general modulation of grooming movements, sending excitation projections into the striatum, and receiving excitatory afferent neurons in the thalamus and amygdala.

The direct output circuits of the **basal ganglia** are particularly significant in compulsive behavioral patterns characterized by serial perseveration and rigidity

[26] [30] [31] [32] [33] [34]. It is possible that the basal ganglia circuitry, which is evolutionarily ingrained in the control of self-grooming in mammals, may also contribute to the maintenance of pathological superstereotypes in humans. For ex: mesocortical-striatal disorders in humans that lead to washing rituals or compulsive self-cleaning to avoid perceived contamination may involve similar mechanisms as observed in self-grooming in rodents. Pharmacological manipulations can effectively modulate grooming behavior in rodents.

The **cerebellum**, which has multiple connections to the basal nuclei, thalamus, cerebral cortex, amygdala, brainstem, and the spinal cord is involved in controlling grooming behavior and coordinating their movements.

Self-care behavior is also modulated by limbic circuits, including the amygdala and hypothalamus (**Figure 2**, **Figure 3**).

The **amygdala** is a limbic brain structure that is involved in regulating the modulation of motivational states such as fear, anxiety, and desire [35]-[40]. Studies have demonstrated a correlation between increased anxiety behavior and decreased amygdala dopamine release in well-groomed selectively bred rats compared to low-groomed rats [24] [29] [30] [31]. The dilated amygdala is an anatomical system that forms a continuum extending from the amygdala to the bed nucleus of the terminal streak (BNST) and to the sheath of the nucleus accumbens. This complex is involved in the regulation of reward and effect. The expanded amygdala contains a medial compartment that includes the medial nucleus of the amygdala (MeA), the medial BNST and a lateral compartment that includes the central nucleus of the amygdala (CeA) and the lateral BNST. Both departments are involved in personal care and seem to work together. For example, stimulation of glutamatergic neurons in the posterior dorsal MeA (MeApd) induces repetitive grooming in mice and suppresses social interaction, whereas stimulation of GABAergic neurons by MeApd inhibits grooming in mice and promotes social interaction. In the lateral amygdala, microinjections of orexin-B into CeA cause a modest increase in the frequency of self-grooming in hamsters [41] [42] [43] [44] [45], collectively confirming a role for both MeA and CeA in modulating self-grooming.

Further investigation is needed to explore the potential roles of indirect amygdala-cortico-striatal networks in grooming. The connection between the striped body and the amygdala helps differentiate between locomotor and sequential self-grooming control associated with basal ganglia circuits and affective state-related self-control modulated by the amygdala (corresponding to limbic chains). However, since the affective state plays a central role in striatal modulation, this contrast may be an oversimplification, and therefore, the functional and anatomical diversity of both the amygdala and striatal regions must be taken into account. For instance, the complex context-dependent modulation of grooming behavior may involve circuits like BLA-CeA-anterior BNST, which mediate stress, anxiety, and conditioned defense, and MeA-posterior BNST circuits projecting to the hypothalamus, responsible for innate social activity and anti-predator behavior [35] [46] [47] [48] [49].

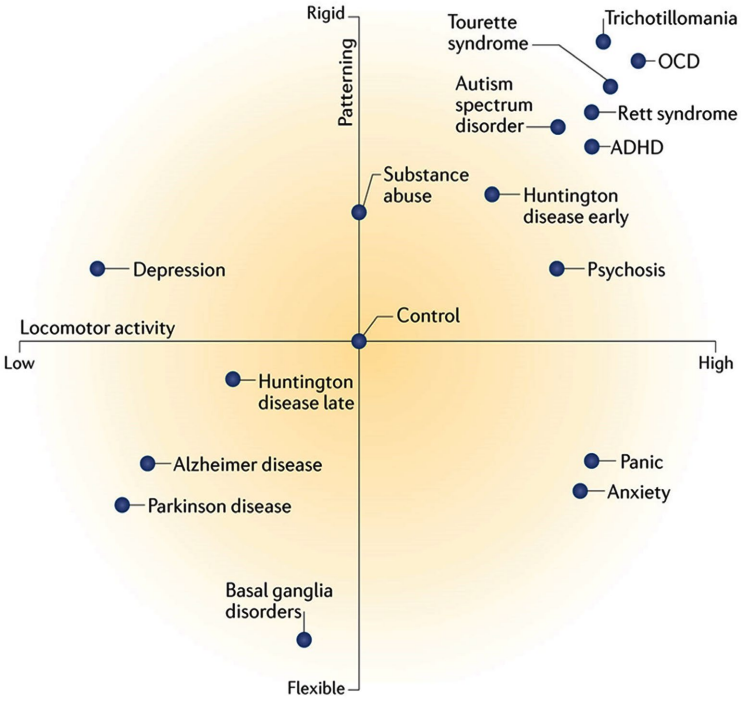

Figure 3. Expected grooming behavior in animal models with neuropsychiatric and neurodegenerative disorders (Kalueff A.V. *et al.*, 2016).

The **hypothalamus** is very important, which is connected to the cerebellum, the amygdala and accordingly, the cortex. At the same time, it is very interesting to connect it with the structures of neuroendocrine regulations, because it is known that the paraventricular nucleus and the dorsal hypothalamus play a big role in self-regulation, since their stimulation leads to strong self-regulation [50] [51] [52] [53] [54].

The hypothalamus, a forebrain area involved in coordinating neural and endocrine regulation of brain function and behavior, also plays a role in regulating self-grooming in rodents [54] [55] [56] [57] [58]. Stimulation or drug injection into the hypothalamus induces intense self-grooming in rats, suggesting that the paraventricular nucleus and dorsal hypothalamus may contribute to this behavior [54]. The paraventricular nucleus projects to MeApd, and glutamatergic neurons in the lateral hypothalamus adjacent to MeApd facilitate repetitive self-grooming in mice [35]. Both CeA and MeA project to their respective regions of the BNST, which serves as the primary connection between the amygdala and the hypothalamus [59] [60]. The amygdala nuclei, particularly MeApd involved in self-care [52], also project to the medial hypothalamus [35].

It seems that the **neocortex** plays an important role in the process of grooming and movement in rats, it directs excitatory processes in the striatum, the processes go from the thalamus to the striatum, then to the brainstem, the spinal cord it also involves basal ganglia and thalamus, all of these parts of the brain are needed for the manifestation of their self-care behavior which is elicited when there is stress or social interactions [35] [50] [51] [61].

The **brainstem**, which is a self-organized chain, is used to initiate self-organized movements and generate basic sequential patterns (such as syntactic chaining). However the brainstem alone is not sufficient to fully implement these patterns (as this requires striatal involvement).

The **basolateral nucleus** (BAN)—projects to the prefrontal cortex, which, along with other cortical regions, in turn projects to the striatum. Although it is known that cortico-striatal connections can modulate grooming behavior [42] [62] [63] [64] [65] (**Figure 2**), the potential functions of indirect amygdala-cortico-striatal networks in body care remain unclear. The connection between the striatum and the amygdala also raises the possibility of distinguishing between locomotor and sequential control of self-grooming (which is associated with basal ganglia circuits) compared to mood-related self-care. However, since affective state is central to the modulation of striatal state, this contrast may be an oversimplification, and therefore the functional and anatomical diversity of amygdala and striatal contours should be considered. For example: complex context-dependent modulation of self-care. may include both BLA-CeA-anterior BNST circuits (which mediate stress, anxiety, and conditioned defense) and MeA-posterior BNST circuits projecting to the hypothalamus (which are responsible for innate social and predator-defence behaviors [66].

7. Neurohormone's Role in Grooming Regulation

Based on different studies, can be concluded that endogenous substances such as neurotransmitters: dopamine, gamma-amino acid, and serotonin and hormones: adrenocorticotropic, vasopressin, oxytocin, prolactin, bombesin, and corticotropic-releasing hormones, as well as various areas of the brain, especially the basal ganglia and hypothalamus, are involved in rodent grooming initiation and regulation [49] [51] [52] [68] [69] [70] [71]. The effects of these hormones on grooming partially rely on the mesolimbic dopaminergic system [55] [72] [73].

Stress-related hormones such as the hypothalamic-pituitary system also mediate self-grooming, **corticotropin**-releasing hormone (CRH) and **adrenocorticotropic** hormone (ACTH), are particularly potent in triggering self-grooming chain processes in rodents [27] [50] [61] [74] [75] [76] [77] [78].

***Role of GABA*:** Gamma-aminobutyric acid (GABA) is involved in the regulation of both anxiety and courtship. The GABA-ergic anxiolytic substance acts on the cephalous-caudal direction and reduces the number of interrupted groomings and the total percentage of incorrect transitions between the grooming phases. Examination the predictive validity of the microstructure of grooming behavior as a marker of anxiety by measuring the effects of two reference GABAergic compounds: the anxiolytic diazepam (0.1 and 0.5 mg/kg ip) and the anxiogenic pentylenetetrazol (5 and 10 mg/kg ip) for the care of mice, shows that the percentage of pattern transitions is not consistent with cephalous-caudal progression and the percentage of aborted grooming episodes and are more reliable behavioral markers of stress that are bidirectionally sensitive to GABAer-

gic anxiogenic and anxiolytic drugs compared to frequency and duration [79].

Another example of rodents with specific mutations showing an aberrant grooming phenotype is mice that lack the GABA-synthesizing enzyme glutamate decarboxylase 1 (GAD1; also known as GAD67) in striatal neurons. These mice exhibit behavioral abnormalities that resemble ASD symptoms, including stereotypical body grooming and impaired spatial learning and social behavior, [34] suggesting that GABAergic striatal output may contribute to behavioral deficits in ASD118. OCD is a common heterogeneous mental disorder characterized by obsessions and compulsions [19] [80]. Obsessions are intrusive, repetitive and persistent unwanted thoughts that are often associated with increased anxiety [19] [80].

Mutant rodents, such as mice lacking the GABA-synthesizing enzyme glutamate decarboxylase 1 (GAD1 or GAD67) in striatal neurons, exhibit abnormal grooming behaviors similar to symptoms of Autism Spectrum Disorder (ASD), including stereotypical body grooming, impaired spatial learning, and social behavior. This suggests that GABAergic striatal output may contribute to behavioral deficits in ASD [13] [14] [81].

D1 and D2 systems: Research on rodent care may provide valuable information about the contribution of dopamine to the initiation, organization, and repetition of motor patterns.

Dopamine, a major modulator in the mesolimbic and nigrostriatal systems, is essential for various physiological functions, including motor skills, the motivational aspects of emotions and behavior, conditioned reflex action, and specific forms of behavior such as grooming [26] [65] [66].

Dopamine is the primary modulator in the nigrostriatal and mesolimbic systems and plays a crucial role in motor function, self-care, and other complex behavior patterns [28] [52] [65] [82] and the main **dopaminergic** sources regulate the sequential processes of self-care in rodents, which are generated in the brain stem of rodents [27] [28] [83].

Stimulation of dopamine D1 receptors in rodents (e.g., systemic administration of D1 agonists) enhances complex behavioral super-stereotyping by excessively producing grooming chains, including particularly rigid stereotypical chains [14] [15] it disappears by activating dopamine **D2** receptors [16] [53]. Systemic administration of D2 receptor agonists, such as Haloperidol, inhibits the stereotypical nature of the grooming sequence [3]. The proper functioning of the nigrostriatal system is crucial for the performance and coordination of complex and consistent grooming behavior. Intra-cerebral microinjection of 6-hydroxy dopamine, cyanic acid, and other neurotoxins in this area disrupts the structure of stereotypical movements associated with damage to dopaminergic receptors.

Mice with heightened neurotransmission in the D1 circuit, and DAT-deficient mice, have demonstrated that the activation of neuronal circuits expressing D1 leads to the generation of excessively stereotypical yet consistently complex grooming patterns [14] and [39].

Sequential super stereotypy induced by the D1 agonist SKF38393 can be prevented through systemic co-administration of the D2 receptor antagonist haloperidol [39]. Similarly, the activation of grooming by SKF83959, a D1 agonist and partial D2 agonist, can be eliminated in mice lacking the D1 gene (not D2) [40]. These findings collectively highlight the significance of maintaining a balance between the D1 and D2 systems of the striated body in the regulation of self-care.

Injections of 6-0HDA were used to disrupt nigrostriatal dopamine projections to deplete striatal dopaminergic inputs. Dopamine depletion in the striatum has been found to impair the efficient completion of syntactic grooming chains. The disruption of the syntactic chain has been correlated with aphagia, which follows after dopamine depletion. The loss of either dopamine projections or striatal intrinsic neurons causes an equivalent disruption of syntactic chains of care [69] [73] [77] [78] [84] and loss of any component of the nigrostriatal system disrupted chain formation to a degree equal to that which occurs with the loss of the entire forebrain.

Neuromedin U: The modulation of sexual behavior in male mice by the anorexigenic peptide neuromedin U (NMU) was investigated, but instead of affecting sexual behavior, NMU administration increased self-grooming. Oxytocin and dopamine in the mesocorticolimbic system were found to be involved in NMU-induced self-grooming behavior, and antagonism of dopamine D2 receptors inhibited this behavior. NMU treatment also affected dopamine levels and D2 receptor expression in the nucleus accumbens. These findings suggest that NMU treatment, in combination with social cues triggering oxytocin release, induces excessive grooming behavior in male mice, with the mesolimbic dopamine system playing a crucial role.

The link with obsessions, compulsions and other medical conditions

Compulsions involve engaging in repetitive actions or thoughts, and it is generally believed that they are performed to alleviate obsessive thoughts. However, the link between obsessions and compulsions is not entirely certain. Compulsions sometimes revolve around personal hygiene, such as self-cleaning or self-care activities like hand washing, as well as behaviors aimed at avoiding perceived contamination from the environment [28] [30] [31] [32] [33] [85]. Various studies, including those involving individuals with OCD syndromes, neuroimaging, clinical genetics, and animal models of repetitive behavior, suggest that dysfunction in the basal ganglia circuitry contributes to these syndromes.

Evidence from studies on individuals with OCD syndromes, including neuroimaging and clinical genetics, suggests that dysfunction in the basal ganglia circuit contributes to these syndromes. Various genetic mutations have been found to affect grooming behavior in rodents, making them useful models for studying OCD symptoms related to self-care, such as compulsive hand washing and hair pulling. Serotonergic drugs used to treat clinical OCD symptoms have

also been shown to reduce aberrant grooming behaviors in mutant mice, indicating a potential role of the serotonergic system in regulating normal and pathological grooming in humans and rodents. Dopaminergic dysfunctions also play a significant role in auto grooming and its microstructure, allowing for the assessment of their involvement in the CNS pathogenesis. Analyzing auto grooming in animal models of CNS disorders with undiagnosed etiopathogenesis, such as Tourette's syndrome and obsessive-compulsive disorder, is of great interest to researchers.

Animal models exhibit specific grooming behavior phenotypes associated with different diseases. These phenotypes can be measured based on grooming activity magnitude (axis X) and sequential patterns (axis Y), ranging from rigid and repetitive grooming to more flexible and non-stereotypical grooming.

Animal models of movement disorders, such as Parkinson's disease and Alzheimer's syndrome, exhibit deficits in grooming due to motor impairments. Auto-grooming assessment is a valuable component of neurological testing in animals to evaluate motor function and coordination.

8. Pharmacological Studies

Various psychotropic drugs (e.g., diazepam) also have effects on grooming, which as a whole is a good tool for analyzing the behavior of this phenomenon in neurology [23] [31] [72].

Pharmacological studies have demonstrated the involvement of **glutamate** in the regulation of self-grooming [46]. For instance, the systemic administration of anti glutamatergic agents such as the NMDA receptor antagonist phencyclidine (PCP) is a well-established experimental method for inducing grooming in rodents [35].

PCP induces generalized hyperlocomotion and other stereotypic behaviors in rodents [79] [86] [87] [88] [89]. Importantly, while PCP increases the duration of experimentally induced self-grooming, it only disrupts the self-grooming sequence when animals are stressed [35], indicating that self-grooming activity and its detailed patterning are differently controlled within the central nervous system (CNS).

Fluoxetine (commercial name Prozac) is a selective inhibitor of serotonin reuptake, reducing the total amount of cephalous-caudal grooming. Amitriptyline, a tricyclic antidepressant with a pronounced sedative effect, has a similar effect on grooming behavior [39] [62] [89].

Substances, that enhance GABAergic tone, such as **benzodiazepines** and allopregnanolone, generally reduce the inclination for self-grooming at non-sedating doses in rodents [69] [84]. Conversely, GABA inhibitors often enhance grooming in rodents and can reverse the anti-grooming effects of GABA-enhancing agents [44] [45] [57] [58] [79]. GABA stimulators and other anxiolytic drugs may suppress stress-induced grooming by attenuating the intensity of anxiogenic stimulus perception, as anxiety-like states can alter rodent grooming and

grooming sequences [51] [53] [89]. The cephalocaudal self-care pattern in rodents is sensitive to GABAergic drugs: Drugs that inhibit GABA signaling typically disrupt the cephalocaudal pattern, while drugs that enhance GABA signaling tend to normalize this response [57] [71] and [86].

Considering the widespread presence of GABA and glutamate in the CNS, region-specific manipulations are necessary to gain further insights into their roles in self-care. For example, the injection of **zolpidem**, a GABA type A receptor agonist (GABAA), into the hamster CEA did not affect orexin B-induced grooming behavior, whereas co-administration of an NMDA receptor agonist potentiated the effect of orexin B [41]. Injection of **muscimol**, a GABA receptor agonist, into the BNST (but not BLA) significantly reduced the grooming response induced by exposure to cat urine [70], suggesting that this area may play a critical role in anxiogenic responses in general, including heightened self-grooming. Administration of **muscimol** into the ventral tegmental potentiated excessive self-grooming induced by α-melanocyte-stimulating hormone [81]. In contrast, treatment with the **NMDA** receptor antagonist memantine improved pathological self-grooming in mice lacking astrocyte-specific excitatory amino acid transporter 2 (GLT1), which exhibit aberrant excitatory transmission at cortico-striatal synapses [90]. Together, these findings indicate the involvement of key central neurotransmitters and their circuits in the regulation of self-grooming.

9. Grooming Behavioral Tests

Different strains of rodents exhibit variations in grooming behavior. For example, low-level allogrooming is observed in BTBR, NF-kB, p-50, and EN2 - / - mouse strains, while the R117X strain displays high-level allogrooming. Cephalocaudal grooming is poorly expressed in the 129S1 strain, whereas it is normally observed in BALB/c and NMRI strains. Self-grooming is highly expressed in the Ninj1 knockout strain, and the SAPAP3-mutant strain also exhibits high levels of self-grooming. The BTRB strain demonstrates high levels of both self-grooming and other repetitive behaviors, while SHANK3 exhibits self-grooming that causes harm to its own body [28] [82] [87].

Behavioral tests conducted on rodents often encounter various challenges. These tests are time-, space-, and labor-intensive, and can be costly. Moreover, their performance tends to be inherently low to moderate [81] [90] [91].

Some rodent behaviors require extended testing periods to detect, while others necessitate specific conditions (e.g., home cage testing) or long-term evaluation. Additionally, behavioral responses to new drugs or genetic mutations can occur spontaneously when animals are unobserved. Behavioral phenomenology, a rapidly advancing field that combines phenomenology and neuroscience, seeks to link behavioral phenotypes to genetic and environmental factors [80] [92] [93].

Currently, such analysis cannot encompass all aspects of self-care, but it holds great potential for improvement through the utilization of multiple cameras, 3D

spatial imaging of various body points, and enhanced integration of IT-based signals. It is likely that even more advanced tools for analyzing grooming activities will become available in the near future. For instance, systems enabling simultaneous detection and integration of different behavioral cues, such as vibration and image, have already enhanced the phenotyping of rodent grooming [91]. As signal detection and behavior recognition capabilities continue to improve, automated grooming analysis and associated behavior assessments may see increased adoption in high-throughput phenotyping [93]. Previously, typical self-service pattern analysis that required days and the involvement of two or three researchers can now be accomplished much more quickly using these new technologies [92].

Methodologically, grooming is caused by exposure to various stressors, such as strong light, water spraying, tail pulling, swimming, spontaneous grooming (induced by a new environment), and immobility. Various behavioral tests are used for grooming research.

- "***Adhesive tape removal***" - Adhesive tape is placed on the body of a rat; the animal feels it and tries to remove it through grooming movements. This test is used to check the ability of grooming movements in rodents.
- "***Social Relationship Test***" - It is used to observe grooming behavior in a social context. In this test, two rodents are placed in one cage and their behavior is observed (allogrooming and self-grooming).
- "***Three-Chamber Test***" - It is also called a sociability camera, used to assess the cognitive skills and grooming of a familiar and foreign rodent (newly introduced). Usually, an animal spends more time studying a newly acquainted animal than a familiar one. This test helps us to identify the lack of sociability in mice and rats. Although the three-chamber test is used for social interaction research. Grooming behavior is naturally manifested in the process of observation and the researcher can do structural and quantitative analysis of this phenomenon [93] [94].
- "***Spray Test***" - In this test, the rodent is sprayed once with water, placed in a transparent Plexiglas chamber and grooming is observed. The spray test is a simple method of triggering grooming under experimental conditions, where water is the trigger for this behavior [95] [96] [97].
- "***Open Field Test***" - This test is used to measure anxiety and research activity. The open field represents a new environment and causes animal stress, and using it in the study of grooming is very informative.
- "***The Elevated Plus Maze (EPM) test***" - Is a test that is considered the "gold standard" for studying anxiety behavior. With this test, the rodent finds itself in a stressful situation and its behavioral reactions are further studied and recorded. Grooming can be induced by anxiety; therefore, this test is also convenient for studying grooming behavior [98] [99] [100].
- "***Predator or Predator Odor Exposure Test***" - In this test, a rat can be used against a mouse, a snake against a rat, or a mouse, and it is also possible to use the smell of any predator. Thus, this test will cause stress grooming with

its characteristic parameters [82] [101].

10. Conclusions

Thus, grooming research has great potential in terms of both zoological and translational medicine. The grooming analysis is an indispensable tool in the neurobiological study of behavior and helps us to create a new, exciting experimental model of various diseases of stress and the nervous system. In turn, the results of fundamental studies of translational medicine based on animal models will make promising achievements in clinical medicine in terms of accurate diagnosis and treatment of a number of unknown ethio-pathogeneses in humans, especially psycho-neurological and other genesis diseases.

The alteration of grooming data and the observation of grooming cycles assume a pivotal role in evaluating the efficacy of pharmacological agents. A comprehensive comprehension of grooming and an insightful analysis of its underlying mechanisms equip us with the tools to effectively manage pathologies of the nervous system. For instance, considering the well-established involvement of dopamine-containing neurons in the initiation and sequencing of movements, the meticulous examination of the regulation and potential dysregulation of the dopaminergic system in various brain disorders becomes an area of particular interest for future research [102].

Prospective investigations could extend their focus to broader networks of molecular factors that interconnect with dopaminergic genes. These genes encode proteins that directly govern dopamine signaling and metabolism, cytoskeletal processes, synaptic release, calcium regulation, adenosine, as well as glutamatergic and GABAergic signaling. By assessing the roles of these genes in rodent grooming behavior and establishing links to genes associated with human brain disorders, a more profound understanding of these intricate processes can be attained [103].

Additionally, the microstructure of auto grooming is sensitive to anxiolytic agents, providing new perspectives for studying anti-anxiety drugs. While anxiolytic pharmacological agents are widely used, they often come with undesirable side effects, drug addiction risks, and social stigma.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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