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## *Neurobiology of rodent self-grooming and its value for translational neuroscience*

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## Neurobiology of rodent self-grooming and its value for translational neuroscience

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### Abstract

Self-grooming is a complex innate behaviour with an evolutionary conserved sequencing pattern and is one of the most frequently performed behavioural activities in rodents. In this Review, we discuss the neurobiology of rodent self-grooming, and we highlight studies of rodent models of neuropsychiatric disorders — including models of autism spectrum disorder and obsessive compulsive disorder — that have assessed self-grooming phenotypes. We suggest that rodent self-grooming may be a useful measure of repetitive behaviour in such models, and therefore of value

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#### Competing interests

The authors declare no competing interests.

#### DATABASES

Simons Foundation Autism Research Initiative gene database: <http://gene.sfari.org/autdb/Welcome.do>

#### SUPPLEMENTARY INFORMATION

See online article: S1 (movie) | S2 (movie) | S3 (table) | S4 (table) | S5 (figure)

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to translational psychiatry. Assessment of rodent self-grooming may also be useful for understanding the neural circuits that are involved in complex sequential patterns of action.

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*Professor John C. Fentress (1939–2015) passed away suddenly after this manuscript was initially submitted. The remaining authors dedicate this Review to him as a tribute to a brilliant scientist, good friend and a true pioneer of neurobiology research.*

Self-grooming in animals is an innate behaviour that is involved in hygiene maintenance and other physiologically important processes, including thermoregulation, social communication and de-arousal<sup>1–6</sup>. It is one of the most frequently observed behaviours in awake rodents and has a patterned, sequential organization with characteristic cephalocaudal progression<sup>7–11</sup> (FIG. 1). Self-grooming is remarkably similar across species in several taxa<sup>1–5</sup>. Humans engage in self-grooming, and this behaviour shows some similarity to that seen in other animals<sup>12,13</sup>. However, human self-grooming behaviour can become pathological, for example, during stressful conditions or in certain neuropsychiatric disorders<sup>7–11,14,15</sup>.

The assessment of rodent self-grooming is potentially useful for translational neuroscience research, as aberrant rodent self-grooming can be related to human disorders in which abnormal self-grooming is a symptom. However, it is important to note that animal self-grooming cannot be considered an exact model of any particular human pathology. Rather, the broader value of rodent self-grooming is as a model of complex repetitive, self-directed and sequentially patterned behaviours. Therefore, rather than viewing rodent self-grooming behaviour as a direct correlate of a particular symptom, it may be best considered as an indirect index of several behavioural phenomena that may be relevant to human brain disorders, including chains of motor action and complex patterning of motor activities. From this broad viewpoint, the analysis of rodent self-grooming may help in understanding the neural mechanisms of hierarchical motor control<sup>14–26</sup> that underlie complex sequential behaviours in general, and may also provide valuable mechanistic insights into their dysregulation.

Neurophysiology, genetics and pharmacology have been used to study this interesting complex behaviour in rodents<sup>14–26</sup>. In this Review, we discuss findings from this work and highlight the potential implications of assessing rodent self-grooming behaviour for understanding human brain disorders. We propose that rodent self-grooming is an important behavioural phenotype that can be used to understand the neural basis of complex action patterns in other species, including humans, in both normal and abnormal conditions. This Review does not discuss heterogrooming (a form of grooming behaviour that is directed towards another animal, which occurs in other contexts, such as maternal, sexual and aggressive or social behaviours), or peripheral and brainstem or spinal coordination mechanisms that are the ultimate targets of the forebrain control networks involved in grooming.

## Neurobiology of rodent self-grooming

### Behavioural complexity

Self-grooming in mice and rats shows a high level of behavioural complexity and organization (*grooming microstructure*)<sup>7,27,28</sup>, which involves a series of individual movements that form functional sequences, including highly stereotyped patterns<sup>9</sup> (FIG. 1a). In the first postnatal days, rodent self-grooming behaviour targets the face and consists of either temporally isolated grooming strokes with the front paws or bouts of strokes with varying amplitude and symmetry. During the following weeks, self-grooming behaviour develops to include symmetrical, double-handed lower amplitude movements and finally matures into the species-typical sequencing of short and long symmetrical and asymmetrical strokes<sup>10</sup>. Thus, in such early stages of development, self-grooming consists of facial grooming alone, but over time comes to include grooming of the entire head, neck and trunk. In addition to displaying the stereotyped grooming that is also present in young animals, adults show more flexible, less stereotyped facial grooming movements<sup>10</sup>.

Mature rodent grooming behaviour consists of specific and highly stereotyped patterns of sequential movements, known as a syntactic chain pattern<sup>7</sup>, which often occurs during the transition between facial and body grooming (FIG. 1a,b). Syntactic chains of self-grooming have features similar to those of other *fixed-action patterns*, such as sexual or aggressive behaviours, in that they are highly stereotyped in order, and, once begun, they proceed to completion without requiring sensory feedback<sup>7</sup>. A typical self-grooming syntactic chain in rodents, which is often embedded in other forms of grooming behaviours, serially links 20 or more grooming movements into four distinct, predictable phases that follow the same cephalocaudal (head-to-body) rule<sup>9,29</sup>. The serial structure of such chains is repetitive and consistent in terms of order and time, so that once the first phase begins, the entire remaining sequential pattern reliably continues through all four phases. This syntactic chain pattern accounts for approximately 10–15% of all observed self-grooming behaviours in rodents, the remainder of which follow less predictable sequential patterning rules (FIG. 1; see Supplementary information S1 (movie))<sup>7</sup>. Self-grooming sequencing, chain initiation and chain completion in rodents can be bidirectionally affected by experimental manipulation, including lesions of the dopamine-containing nigrostriatal tract, administration of various dopaminergic drugs, genetic mutations and psychological stress<sup>7</sup>. The syntactic chains are usually interspersed with more flexible ‘non-chain’ grooming (that is, flexibly ordered mixtures of strokes, licks or scratches that are not components of syntactic chains), which accounts for approximately 85–90% of all grooming behaviours<sup>7</sup> (see Supplementary information S2 (movie)). Ethologically based analyses of grooming behaviours, including both chain and non-chain bouts, are widely used in neurobiological research to assess their global adherence to the cephalocaudal rule<sup>7</sup>. Correct and incorrect cephalocaudal transitions between stages can be studied in this way (FIG. 1c), along with interruptions in grooming bouts (as an index of disturbed self-grooming) and their regional distribution over the body<sup>7,28,30</sup>. Such analyses demonstrate the high sensitivity of grooming sequencing to genetic, pharmacological and psychological challenges<sup>7,27,28,30–33</sup>.

## Neural circuitry of self-grooming

Because of its highly patterned nature, grooming is particularly suitable for studying how various neural circuits regulate both the key aspects — motor and sequencing — of this behaviour<sup>34</sup>. Studies of rats decerebrated at successively lower levels of the neuraxis have demonstrated that rats that underwent mesencephalic decerebration, in which the midbrain is intact, have a normal sequential pattern of self-grooming chains, although such animals have difficulty in completing the full pattern<sup>35,36</sup>. By contrast, a gradual degradation of the sequential pattern itself is seen in rats that have been decerebrated at more caudal (that is, metencephalic and myelencephalic) levels, suggesting that the brainstem circuitry is necessary for the execution of fully patterned grooming sequences<sup>35,36</sup> (FIG. 2).

Within the forebrain, circuits that incorporate the basal ganglia and allied nuclei, including the striatum, globus pallidus, substantia nigra, nucleus accumbens and subthalamic nucleus, have been strongly implicated in hierarchical motor control and sequencing of behaviour, including self-grooming. The striatum is the main input region of the basal ganglia. Striatal circuits are involved in learning, motivation and motor sequencing. For example, the basal ganglia<sup>37,38</sup> and, in particular, the striatum, are required for the execution of full sequential patterns of grooming chains and other types of sequential behaviour in mice and rats<sup>18,39,40</sup> (FIG. 2). Lesions of the striatum result in a permanent deficit in the ability to complete sequential syntactic self-grooming chains<sup>34</sup> (FIG. 1b). Extensive work using localized striatal lesions has shown that it is the anterior dorsolateral region of the striatum that is essential for this normal grooming behaviour. Damage to this striatal region impairs the completion of (but not the ability to initiate) syntax patterns of grooming movements<sup>34</sup>. Rats with such striatal lesions completed only ~50% of the syntactic chains, a completion rate that is similar to that of rats with full mesencephalic decerebration (that is, transection above the midbrain)<sup>34</sup>, whereas control rats completed ~90% of the chains. Thus, similar deficits of pattern completion are produced by anterior dorsolateral striatal lesions and by decerebration, but both mesencephalic and pontine decerebrates can still produce the basic sequential self-grooming pattern<sup>34</sup>.

These results suggest that the essential pattern generator for syntactic chains is in the brainstem, and that the dorsolateral striatum may act as a forebrain controller to coordinate the normal completion of the chain pattern. By contrast, lesions that affect the major output nuclei of the basal ganglia, including the ventral pallidum and globus pallidus, disrupt the movements that are required for grooming but not the syntax of self-grooming<sup>34</sup>. These studies suggest that distinct striatal pathways may regulate self-grooming activity and its patterning and sequencing. Neurons in the dorsolateral striatum and the substantia nigra pars reticulata also show distinct spiking patterns during different types of grooming. For example, some dorsolateral striatal neurons that are active during syntactic grooming sequences are unresponsive during kinematically similar movements that occur during flexible grooming<sup>41</sup>. Because striatal neurons can code various types of naturally sequenced behaviours, it is likely that the basal ganglia have a crucial role in the control of sequential movement not only in self-grooming but also in the complex natural patterns of other sequenced behaviour<sup>41–43</sup>.

Lesions made in the neocortex or in the cerebellum produce timing deficits and abnormalities in the individual movements of self-grooming without affecting the sequential pattern of grooming chains<sup>44</sup>. Other manipulations in the cerebellum also affect self-grooming. For example, electrical stimulation of the cerebellum elicits self-grooming in rats<sup>45</sup>, whereas Lurcher mutant mice, which have cerebellar degeneration, display reduced duration, but unaltered sequencing, of self-grooming compared with wild-type mice<sup>46</sup>. Given that the striatum, the neocortex and the cerebellum are directly and indirectly interconnected with one another in movement-control networks, this difference in the amount versus the patterning of self-grooming suggests that the striatum and its associated neural pathways are particularly important for the sequencing of grooming patterns<sup>44</sup>. This conclusion is in accord with other evidence supporting the importance of the striatum-based circuits in sequential behaviours in general<sup>42,43</sup>.

Self-grooming behaviour is also modulated by the limbic circuitry, including the amygdala and the hypothalamus (FIG. 2). The amygdala is a limbic brain structure that is involved in the regulation of modulating motivational states, such as fear, anxiety and desire<sup>47</sup>. Studies have demonstrated correlations between increased anxiety-like behaviour and reduced dopamine release within the amygdala in selectively bred high-grooming versus low-grooming rats<sup>48</sup>. The extended amygdala is an anatomical system that forms a continuum stretching from the amygdala, to the bed nucleus of the stria terminalis (BNST), and to the nucleus accumbens shell. This complex is involved in the regulation of reward and affect. The extended amygdala contains a medial division, which includes the medial nucleus of amygdala (MeA) and the medial BNST, and a lateral division, which includes the central nucleus of the amygdala (CeA) and the lateral BNST. Both divisions are implicated in self-grooming and seem to act together. For example, stimulation of glutamatergic neurons in the posterior dorsal part of the MeA (MeApd) induced repetitive self-grooming in mice and suppressed social interaction, whereas stimulation of GABAergic neurons of the MeApd inhibited mouse self-grooming and promoted social interaction<sup>47</sup>. Within the lateral division of the extended amygdala, microinjections of orexin-B into the CeA evoke moderate increases in grooming frequency in hamsters<sup>49</sup>, collectively supporting the role of both the MeA and the CeA in modulating self-grooming.

Further study is required to obtain a full understanding of amygdala-related grooming circuitry. For example, in addition to its connections to the striatum, the amygdala — primarily its basolateral nucleus (BLA) — projects to the prefrontal cortex, which together with other cortical regions in turn projects to the striatum. Although it is known that corticostriatal connections can modulate self-grooming behaviour<sup>50</sup> (FIG. 2), the potential functions of indirect amygdalo-corticostriatal networks in grooming remain to be investigated. The connectivity between the striatum and the amygdala also raises the possibility of a distinction between the locomotor and sequencing control of self-grooming (that is linked to basal ganglia circuits) versus self-grooming related to affective states (that is modulated by the amygdala-related limbic circuits). However, because affective state is central to striatal state modulation, this contrast may be an over-simplification, and therefore the functional and anatomical diversity of both amygdala circuits and striatal circuits must be considered. For example, complex context-specific modulation of grooming behaviour may involve both the BLA–CeA–anterior BNST circuits (that mediate stress, anxiety and

conditioned defence) and the MeA–posterior BNST circuits projecting to the hypothalamus (that are responsible for innate social and predator-defence behaviours)<sup>47,51</sup>.

The hypothalamus — a forebrain region that coordinates neural and endocrine regulation of brain functions and behaviour — is another limbic region that has been implicated in the regulation of rodent self-grooming<sup>52</sup>. Local electrical stimulation or injection of a wide range of drugs in the hypothalamus evokes robust self-grooming in rats, suggesting that the paraventricular nucleus and the dorsal hypothalamus may be part of a specific region that is responsible for grooming<sup>52</sup>. The paraventricular nucleus projects to the MeApd<sup>52</sup>, and glutamatergic neurons within the lateral hypothalamic area adjacent to the MeApd contribute to repetitive self-grooming in mice<sup>47</sup>. Both the CeA and MeA project to respective divisions of the BNST — the main connector between the amygdala and the hypothalamus<sup>53,54</sup>. Nuclei of the amygdala, most notably the MeApd (that is implicated in self-grooming<sup>52</sup>), also project to the medial hypothalamus<sup>47</sup>. Finally, the hypothalamic–pituitary system has now been implicated in the modulation of self-grooming, as several hypothalamic and pituitary hormones (including the stress-related peptides corticotropin-releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH)) are known to induce self-grooming<sup>55–58</sup> (see Supplementary information S3 (table)). The effects of these hormones on grooming are partly dependent on the mesolimbic dopaminergic system<sup>59–61</sup> (as we emphasize below). Collectively, this evidence indicates that the hypothalamus (and its connections to the pituitary) is an important brain region that incorporates neural and endocrine regulation of self-grooming<sup>2,62</sup>.

### Pharmacological modulation of self-grooming

Pharmacological manipulations can potently modulate rodent self-grooming. Dopamine, which is a major modulator in the nigrostriatal and mesolimbic systems, is critical for locomotor function, self-grooming and other complex patterned behaviours<sup>29,34,40,44</sup>. In rodents, systemic administration of dopamine D1 receptor (D1) agonists amplifies complex behavioural super-stereotypy, leading both to excessive production of self-grooming chains, and to more rigid self-grooming chains<sup>8,63,64</sup>. Systemic co-administration of the dopamine D2 receptor (D2) antagonist haloperidol prevents sequential super-stereotypy that is induced by the D1 agonist SKF38393 (REF. 64), and the activation of grooming by SKF83959, a D1 agonist and D2 partial agonist, is eliminated in knockout mice lacking the D1 (but not the D2) gene<sup>65</sup>. Collectively, these results illustrate the importance of a balance between the D1 and the D2 systems of the striatum in the regulation of self-grooming.

Striatal circuits can also be characterized in terms of the compartmental architecture of the striatum. Within the striatum of humans and other mammals, chemically specialized macroscopic zones known as striosomes (‘striatal bodies’) form a distributed labyrinthine system within the large volume of the striatum that constitutes the extra-striosomal matrix. This architecture is known as the striosome-matrix architecture, which governs the distribution of nearly all neurotransmitters and their receptors as well as the relative distributions of projection neurons and interneurons in the striatum<sup>66</sup>. Studies have shown that, following dopaminergic challenge, striosomes are strongly activated and express early response genes that code for transcription factors, and that this heightened striosomal



activation is highly correlated with increased repetitive behaviours, including self-grooming, in both non-human primates and rodents<sup>42,67–70</sup>.

Pharmacological studies have shown that glutamate is also involved in the regulation of self-grooming<sup>71</sup>. For example, the systemic administration of anti-glutamatergic agents, such as an NMDA receptor antagonist phencyclidine (PCP), is a well-established experimental method for inducing grooming in rodents<sup>72</sup>. In addition, PCP induces generalized hyperlocomotion and other stereotypic behaviours in rodents<sup>73–75</sup>. Notably, although PCP increases the duration of experimentally evoked self-grooming, it disrupts the sequencing of self-grooming only when the animals are under stress<sup>72</sup>, further indicating that self-grooming activity and its detailed patterning are controlled differently by the CNS.

GABAergic neurotransmission also contributes to the regulation of self-grooming. Drugs that enhance GABAergic tone, such as benzodiazepines and allo-pregnanolone, generally reduce rodent self-grooming at non-sedative doses<sup>76–78</sup>. By contrast, GABA-inhibiting drugs often increase grooming in rodents and can also reverse the anti-grooming effects that are produced by GABA-enhancing agents<sup>76,77</sup>. The GABAergic system is also a key modulator of stress and anxiety-related behaviours in rodents<sup>32,79</sup>. Drugs that enhance GABAergic tone exert anxiolytic effects and may be useful as augmentation agents for the treatment of obsessive compulsive disorder (OCD)<sup>80</sup>. Thus, these GABA-enhancing drugs and other anxiolytic drugs may suppress stress-induced grooming through attenuating the intensity of the perception of anxiogenic stimuli<sup>81</sup>, as anxiety-like states alter rodent self-grooming and its sequencing<sup>28,30,82</sup>. The cephalocaudal patterning of rodent self-grooming is sensitive to GABAergic drugs: drugs that inhibit GABA signalling generally disorganize cephalocaudal patterning and drugs that enhance GABA signalling tend to normalize this response<sup>32,76,83</sup>.

Given the ubiquity of GABA and glutamate in the CNS, region-specific manipulations are required to provide further insights into their role in grooming. For example, the injection of the GABA type A receptor (GABA<sub>A</sub>) agonist zolpidem into the hamster CeA did not affect orexin B-evoked grooming behaviour, whereas co-infusion of an NMDA receptor agonist potentiated the effect of orexin B<sup>49</sup>. Injection of the GABA<sub>A</sub> agonist muscimol into the BNST (but not into the BLA) strongly reduced the self-grooming response that is evoked by cat urine exposure<sup>81</sup>, suggesting that this region may be crucial for anxiogenic responses in general, including increased self-grooming. Administration of muscimol to the ventral tegmental area potentiates the excessive self-grooming behaviour that is evoked by  $\alpha$ -melanocyte-stimulating hormone<sup>84</sup>. By contrast, treatment with an NMDA receptor antagonist, memantine, ameliorates pathological self-grooming in mice that lack the astrocyte-specific excitatory amino acid transporter 2 (also known as GLT1), which display aberrant excitatory transmission at corticostriatal synapses<sup>85</sup>. Taken together, this evidence implicates key central neurotransmitters and their circuits in the regulation of grooming.

## Self-grooming in CNS disorders

Self-grooming in rodents can be used to model normal or pathological human grooming behaviours<sup>86</sup>, but study of this behaviour also has a much broader value, as it can be relevant



to the neurobiology of complex, repetitive and sequentially patterned behaviours<sup>6,7,24</sup>. Different aspects of self-grooming in rodents can be used to mimic phenotypes across a range of human conditions (FIG. 3), only some of which manifest themselves as aberrant grooming. In line with recently introduced research domain criteria (RDoC)<sup>87,88</sup>, we take a dimensional approach and discuss the dysregulation of rodent self-grooming and its value for modelling dimensions of human psychopathology that may cross traditional diagnoses.

### Autism spectrum disorder

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with CNS aetiology and complex symptoms, including difficulties with communication, repetitive behaviours and social deficits<sup>89–92</sup>. There is considerable interest in developing experimental animal models of ASD<sup>90,91,93</sup>. Because self-grooming episodes in rodents are thought to recapitulate pathological repetitive behaviours (behavioural perseveration), strains of rodents that exhibit these phenotypes have been investigated, with the goal of identifying neural circuits and genes relevant to ASD<sup>33,90,94</sup>. We discuss rodent self-grooming as a measure of behavioural perseveration rather than as a specific model of an ASD phenotype. Notably, many of the mouse strains discussed here also display other phenotypes that are relevant to ASD (for example, they also show non-grooming behavioural perseverations and/or deficits in other relevant domains, such as social impairments and anxiety).

The inbred BTBR  $T^+ Itpr^{3fl}/A$  (BTBR) mouse strain, which exhibits agenesis of the corpus callosum, displays several aberrant behaviours that resemble symptoms of ASD, including social deficits, anxiety and general behavioural inflexibility<sup>19,95,41–43</sup>. Peer rearing with a different ('non-ASD') strain improved social deficits in BTBR mice but did not improve their repetitive self-grooming<sup>96</sup>, raising the possibility that different ASD behavioural domains may be regulated by distinct brain mechanisms. However, increased self-grooming in these animals can be corrected pharmacologically. For example, cholinergic agents (which may be useful in correcting postulated cholinergic deficits in ASD<sup>97,98</sup> and/or some of its clinical symptoms<sup>99</sup>) reduce self-grooming<sup>19</sup> and other ASD-like behaviours<sup>100</sup> in BTBR mice. Furthermore, repetitive self-grooming behaviour in BTBR mice is rescued by the inhibition of glutamatergic metabotropic mGluR5 receptors<sup>90,101</sup> and by the stimulation of NMDA receptors by D-cycloserine<sup>102</sup> (which has also been shown to ameliorate some behavioural deficits in individuals with ASD<sup>103,104</sup>). Environmental enrichment reduces the duration, but not the rigid patterning, of abnormal self-grooming in BTBR mice<sup>33</sup>. The ability to modulate the quantity (amount) and the quality (degree of sequencing) of self-grooming in these mice by distinct interventions raises the possibility that there are also distinctions between these different aspects of self-grooming behaviour at the level of circuits and molecular pathways. Consistent with the goal of defining psychiatric diseases as circuit disorders<sup>105,106</sup>, this work further emphasizes the value of a nuanced understanding of grooming phenotypes, including self-grooming, in preclinical biological psychiatry research.

The genetic mechanisms underlying ASD have been unclear to date, owing to its highly polygenic nature. Currently, the number of genes associated with ASD is estimated to be

~700, according to the Simons Foundation Autism Research Initiative gene database. Individuals with ASD have heterogeneous behavioural and neuromorphological phenotypes<sup>91,106–109</sup>. Three examples are used here to illustrate how assessing self-grooming in transgenic mice can be useful in investigating the role of particular genes associated with autism. *SHANK1* (SH3 and multiple ankyrin repeat domains 1), *SHANK2* and *SHANK3* encode postsynaptic scaffolding proteins that are crucial for synaptic function in the brain<sup>110</sup>, and mutations in these genes are strongly implicated in ASD. In addition to exhibiting ASD-like social deficits and repetitive behaviours, mice with mutations in different *Shank* genes show aberrant self-grooming phenotypes<sup>111</sup> (TABLE 1). For example, *Shank1*<sup>+/-</sup> (and, to a lesser extent, *Shank1*<sup>-/-</sup>) mice demonstrate mildly increased self-grooming behaviour as adults, but not as juveniles<sup>21</sup>. Female, but not male, *Shank2*<sup>-/-</sup> mice lacking exon 7 show increased duration of self-grooming bouts<sup>16</sup>, and male *Shank2*<sup>-/-</sup> mice lacking exons 6 and 7 spend more time engaged in self-grooming during a novel object recognition (but not during the open field) test<sup>112</sup>. Increased duration of self-grooming bouts in *Shank3*<sup>-/-</sup> mice has also been reported by several groups<sup>89,113,114</sup>. Taken together, these findings establish a link between disruptions in *Shank* genes, aberrant synaptic function in the brain and ASD-related behaviours in mice<sup>88,106,107,111</sup>, suggesting the *Shank*-mutant mice, and their self-grooming phenotypes in particular, are good models of ASD.

Ephrin A ligands and ephrin A receptors are strongly implicated in neurodevelopment<sup>115</sup>. Ephrin A ligands are membrane-anchored cellular proteins that bind to ephrin A receptors, members of the receptor tyrosine kinase superfamily. During development, ephrin A-mediated signalling modulates neuronal differentiation and synaptic plasticity<sup>115</sup>. Because ASD is a neurodevelopmental disorder, ephrin A ligands and their receptors may be relevant to ASD and modelling its pathogenesis in animals<sup>115</sup>. For example, mice that lack both the ephrin A2 and the ephrin A3 receptors display robust repetitive self-grooming in addition to motor retardation, increased prepulse inhibition and social deficits (impaired social interaction and preference), thereby paralleling in their phenotypes some of the clinical symptoms of ASD<sup>115</sup>. The search for novel molecular anti-ASD drug targets is a recognized priority<sup>19,93,106,116,117</sup>, and the utility of grooming-based analyses for exploring novel candidate pathways of this disorder (for example, ephrin A receptor agonists) continues to emerge.

Another example of rodents with specific mutations displaying an aberrant self-grooming phenotype are mice lacking the GABA-synthesizing enzyme glutamate decarboxylase 1 (GAD1; also known as GAD67) in striatal neurons. These mice display behavioural abnormalities that resemble symptoms of ASD, including stereotypic grooming and impaired spatial learning and social behaviour<sup>118</sup>, suggesting that GABAergic output from the striatum might contribute to behavioural deficits in ASD<sup>118</sup>.

A deletion on human chromosome 16p11.2, spanning approximately 30 genes, is associated with ASD and other neurodevelopmental disorders<sup>119–121</sup>. Notably, mice heterozygous for a deletion of the syntenic region on chromosome 7F3 (16p11<sup>+/-</sup> mice) show reduced self-grooming behaviour, but the mice also display hyperactivity and behavioural perseverations, such as increased circling<sup>108</sup>. 16p11<sup>+/-</sup> mice also have increased numbers of striatal medium spiny neurons expressing the dopamine D2 receptor, fewer cortical neurons expressing D1

dopamine receptors, and synaptic defects indicating abnormal basal ganglia circuitry<sup>108</sup>. The behavioural phenotype of these mice is of particular note, because the decreased self-grooming is observed alongside increased non-grooming stereotypies<sup>108</sup>, thereby suggesting further distinctions between the activity and patterning aspects of grooming (see TABLE 1 and Supplementary information S4 (table) for more information on genetic models of mouse self-grooming). Finally, studying self-grooming improves the development of animal models of ASD, because when other ASD-like phenotypes are present, the co-occurrence of a self-grooming phenotype as a measure of repetitive behaviour considerably strengthens the validity of the models.

### Disorders of the basal ganglia

Excessive self-grooming is a feature of some forms of OCD<sup>24,86</sup> and related illnesses, such as body dysmorphic disorder, excoriation (compulsive skin-picking) and trichotillomania (compulsive hair-pulling)<sup>92</sup>. Studying aberrant self-grooming in rodents may therefore be relevant to modelling such conditions, and may also be useful for modelling the OCD-spectrum disorders that, although they are not associated with abnormal self-grooming, are characterized by excessive repetitiveness of behavioural actions<sup>122,123</sup>.

OCD is a common heterogeneous psychiatric disorder that is characterized by obsessions and compulsions<sup>124,125</sup>. Obsessions are intrusive, recurrent and persistent unwanted thoughts, and are often associated with elevated anxiety<sup>124,125</sup>. Compulsions include a range of repetitive behaviours or thoughts. A conventional view is that these are performed to relieve obsessions<sup>124</sup>, but this link between obsessions and compulsions is not certain. Compulsions are sometimes focused on aspects of personal hygiene, which can involve self-cleaning or self-grooming behaviours (such as hand-washing), and behaviours to avoid perceived contamination from the individual's surroundings<sup>44,86</sup>. Evidence from studies of individuals with OCD syndromes, including neuroimaging and clinical genetics, and from studies of a wide range of animal models of repetitive behaviour<sup>68</sup>, has suggested that basal ganglia-related circuit dysfunction contributes to these syndromes.

A growing number of genetic mutations have been shown to affect self-grooming behaviour in rodents<sup>68</sup> (TABLE 1; see Supplementary information S4 (table)). Some of these may be useful in modelling self-grooming-related symptoms of OCD, including compulsive hand-washing<sup>19,22,25</sup> and obsessive hair-pulling<sup>126–128,129</sup> (TABLE 2). For example, serotonergic drugs that are effective in treating some symptoms of clinical OCD<sup>71</sup> are also successful in reducing aberrant self-grooming phenotypes in some of these mutant mice (TABLE 3). Such findings support the value of rodent self-grooming behaviours in mimicking human OCD. They also raise the possibility that the serotonergic system contributes to the regulation of normal and pathological grooming in both humans and rodents. Although direct support for this notion remains elusive<sup>71</sup>, clinical and experimental evidence continues to implicate serotonergic function in various OCD-like symptoms<sup>130–136</sup>.

Mutations in *SAPAP3*, which encodes synapse-associated protein 90/postsynaptic density protein 95-associated protein 3, have been implicated, though only weakly, in OCD and self-grooming disorders, such as pathologic skin picking, nail biting and hair pulling<sup>122,137,138</sup>. *SAPAP3* binds to *SHANK3*, another post-synaptic scaffolding protein that, as discussed

above, is linked to ASD<sup>44</sup>. In rodents, SAPAP3 is primarily expressed in neurons in the striatum — a key brain region that is involved in the control of self-grooming. *Sapap3*<sup>-/-</sup> mice display robust increased self-grooming that is rescued by the re-expression of *Sapap3* in the striatum<sup>40,139</sup>. Because *Sapap3* is expressed in striatal glutamatergic synapses, these findings suggest that excitatory neurotransmission in this region is important for the regulation of normal self-grooming behaviour<sup>139</sup>. Interestingly, although *Sapap3* deletion reduces corticostriatal synaptic transmission, it does not affect thalamostriatal activity<sup>139</sup>, providing an excellent opportunity to use the abnormal grooming phenotype of the *Sapap3*<sup>-/-</sup> mice to dissect the role of thalamostriatal versus corticostriatal circuits in mediating excessive repetitive behaviours in individuals with OCD. The over-grooming phenotype observed in the *Sapap3*<sup>-/-</sup> mice can be rescued by optogenetic stimulation of the corticostriatal pathway originating in the orbitofrontal cortex<sup>39,68,140,141</sup>. The mechanism underlying this rescue seems to involve striatal high-firing interneurons (that are impaired in this genetic mouse model), directly implicating intrastriatal network activity in the aetiology of the compulsive grooming behaviour. Furthermore, repeated daily stimulation of a nearby part of the orbitofrontal cortex in wild-type mice can evoke a prolonged increase in self-grooming behaviour<sup>15</sup>. These results emphasize the importance of corticostriatal circuits, and potentially intrastriatal microcircuits, in the control of self-grooming in rodents, which may also be relevant to modelling compulsions in individuals with OCD.

Tourette syndrome is another common, highly heritable, childhood-onset neuropsychiatric disorder that is characterized by motor and phonic tics<sup>114,142,127</sup>. This syndrome is frequently comorbid with OCD and attention deficit hyperactivity disorder (ADHD), and can be accompanied by affective disorders, such as anxiety and depression<sup>92,143</sup>. Albeit related to OCD and grooming disorders (such as trichotillomania), Tourette syndrome differs from them genetically and phenotypically<sup>142,144–146</sup>. Owing to its complex repetitive nature, rodent self-grooming behaviour is a logical phenotype to investigate in putative models of Tourette syndrome<sup>29</sup>, especially given that the nigrostriatal dopaminergic system has been implicated in sequential stereotypy of behaviour, which manifests itself as inflexible actions or stereotyped 'rigid' thought in individuals with OCD, individuals with Tourette syndrome, or individuals with both OCD and Tourette syndrome. Therefore, rodents with abnormal dopaminergic signalling can be good candidates for modelling aspects of these disorders<sup>29,147</sup>.

Dopamine transporter (DAT)-deficient mice, which have elevated levels of dopamine, exhibit more stereotyped and predictable syntactic grooming sequences than their wild-type counterparts, with fewer disruptions of syntactic patterns and a sequential 'super-stereotypy' in the complex fixed-action patterns<sup>29</sup>. Dopamine receptor subtypes may mediate different effects of dopamine on self-grooming phenotypes<sup>8,148</sup>. Mutant mice that lack dopamine D1A receptors (TABLE 1) exhibit shorter self-grooming bouts and more disrupted, incomplete sequential patterns<sup>148</sup>. This phenotype suggests that dopamine D1A receptors can specifically modulate the sequencing of grooming behaviour in rodents, which supports findings from human studies suggesting that dopamine receptor subtypes have distinct roles in patients with Tourette syndrome and related basal ganglia disorders<sup>29</sup>. In addition, transgenic mice that express a form of cholera toxin that potentiates neurotransmission

selectively within corticolimbic D1-expressing neurons (D1 neurons) exhibit elevated self-grooming, as well as various juvenile-onset tics, and so mimic aspects of comorbid OCD and Tourette syndrome<sup>149</sup>. Similarly, mutations in the histidine decarboxylase gene (*HDC*) have been implicated in Tourette syndrome, and *Hdc*<sup>-/-</sup> mice display tic-like behaviours that recapitulate certain aspects of Tourette syndrome, including stereotypic self-grooming<sup>150</sup>.

Rodents with impaired motor behaviour and impaired motor sequencing (such as pathologically reduced self-grooming) can also be useful for understanding basal ganglia disorders in general (TABLE 1). For example, the weaver (*wv/wv*) mouse possesses a naturally occurring mutation in the *Girk2* gene that encodes a G protein-activated inwardly rectifying potassium ion channel<sup>30,31</sup>. This mutation markedly affects cerebellar and striatal pathways<sup>151–154</sup> (crucial for motor performance), resulting in an aberrant self-grooming phenotype that consists of more frequent, but shorter, grooming bouts with smaller forelimb strokes and less complete sequences<sup>30,31</sup>. Because they display deficits in the two critical CNS circuits, the context- and age-specific neurological defects in *wv/wv* mice provide a useful tool for examining how the two systems control self-grooming during development<sup>155</sup>. For example, although the mutant mice initially spend less time self-grooming, after day 15 they initiate more frequent, briefer grooming bouts, which are more likely to be associated with striatal control of sequencing<sup>155</sup>. Taken together, these models illustrate the important role of the basal ganglia in the modulation of normal and pathological self-grooming behaviour in rodents, thereby potentially offering translational insights into human basal ganglia disorders.

### Other disorders

It has long been known that acute stressors (for example, exposure to a novel environment or to predators) can potently modulate self-grooming, often increasing the frequency and/or total duration of bouts<sup>1,28,95,156,157</sup> and inducing displacement activity<sup>3,4</sup>. In addition, stressors also result in disorganized grooming patterning<sup>7</sup> by impairing cephalocaudal progression (for example, increasing incorrect transitions that do not follow this pattern), evoking more incomplete bouts, causing more interruptions in bouts of self-grooming, and by disrupting its regional distribution (changing how different parts of the body are being groomed)<sup>7,27,31,158</sup>. High chronic baseline anxiety in certain mouse and rat strains is often accompanied by increased self-grooming and disorganized patterning<sup>7,27,159</sup>, whereas anxiolytic treatments tend to reduce rodent self-grooming activity and normalize its sequential organization<sup>7,28,32,76</sup>. The neurobiological bases of the interaction between stress and self-grooming remain poorly understood, but the brain regions that are involved in affect, especially the amygdala, are likely to be involved (FIG. 2). As stress and anxiety seem to modulate rodent self-grooming, it is possible that abnormal grooming behaviour could be used as a measure of stress or anxiety in various experimental models and tests<sup>7</sup>.

Motor deficits are a feature of major neurodegenerative disorders, and as a complex patterned behaviour, self-grooming is a logical candidate behaviour to assess such deficits in rodent models. Parkinson disease, which produces debilitating impoverishment of voluntary movement, is neuropathologically characterized by the presence of Lewy bodies, which are mostly composed of  $\alpha$ -synuclein<sup>160</sup>. The A53T missense mutation in the  $\alpha$ -synuclein gene

is strongly implicated in the pathogenesis of parkinsonian states<sup>161</sup>. Transgenic mice that express the human A53T variant under the control of the mouse prion promoter display progressive motor and cognitive deficits, including impaired grooming that is observed as early as 1–2 months old<sup>160</sup>, before the onset of spatial memory deficits (at 6–12 months) or abnormal gait (at 12 months). Combined with aberrant synaptic neurotransmission, behavioural analyses of self-grooming in this mouse strain may therefore be useful for assessing novel therapeutic interventions for Parkinson disease<sup>160</sup>.

Several rodent models of other neurodegenerative diseases also display aberrant self-grooming phenotypes, including recently developed rodent models of Huntington disease<sup>162–167</sup>, familial Danish dementia<sup>168</sup>, Krabbe disease<sup>169</sup> and other types of neurodegeneration<sup>170,171</sup>. Collectively, these studies illustrate the utility of self-grooming phenotypes for modelling various neurodegenerative disorders and dissecting their pathobiological mechanisms. Interestingly, aberrant hyper-grooming is observed in the early stages of the disease in a rat model of Huntington disease that is induced by the striatal injection of quinolinic acid<sup>164</sup>, and impaired grooming in A53T-mutant mice appears before parkinsonian-like cognitive or gait deficits<sup>160</sup>. These observations raise the possibility that altered self-grooming may represent an early behavioural hallmark<sup>162,169,172</sup> in these disease models, although this remains to be tested.

## Novel approaches and future directions

Recognizing the importance of neuromorphological endophenotypes related to brain disorders<sup>173,174</sup>, it is logical to apply similar approaches and imaging techniques to rodents with aberrant self-grooming phenotypes. For example, using functional MRI, decreased fronto-cortical, occipital and thalamic grey matter volume and decreased cortical thickness have been detected in hyper-grooming BTBR mice compared with a low-grooming control C57BL/6J mouse strain<sup>175</sup>. In addition, diffusion tensor tractography has confirmed callosal agenesis and impaired hippocampal commissure formation in BTBR mice, whereas resting-state brain activity using cerebral blood volume weighted fMRI revealed reduced corticothalamic function<sup>175</sup>.

Given the complexity and polygenic nature of most brain disorders, research is increasingly focused on identifying sets of genes that contribute to several CNS disorders<sup>176</sup>. For example, although repetitive behaviours (including self-grooming) and increased anxiety are both observed in OCD<sup>177,178</sup>, most clinical and animal studies examine the genetic and physiological correlates of these two behavioural domains separately<sup>179–181</sup> (see also REFS 182,183). Applying large-scale bioinformatics and pathway analyses to complex behavioural endophenotypes and the interactions between them, rather than targeting only individual endophenotypes (for example, assessing genes that are related to pathologically increased self-grooming and the dysregulation of the neural circuits involved in controlling complex movements in a single study), can markedly enrich the landscape of genes related to neuropsychiatric disorders<sup>184</sup>, including those involved in the regulation of self-grooming-related behaviours.



Optogenetic manipulations are a useful tool for understanding the circuits that are involved in rodent self-grooming. As noted above, repeated (but not acute) stimulation of the medial orbitofrontal cortex-ventromedial striatum pathway in mice can trigger pathological self-grooming that lasts for weeks, a condition that can be reversed by the chronic administration of the selective serotonin reuptake inhibitor fluoxetine<sup>15</sup>. Stimulating the nearby orbitofrontal cortex (or its intrastriatal terminals) can block compulsive self-grooming in *Sapap3*<sup>-/-</sup> mice<sup>39</sup>. These findings are important, as they provide strong experimental evidence for circuit-level control of repetitive episodes of grooming. Optogenetic approaches to modulate grooming cannot yet be translated to the clinic, but these studies suggest that future circuit modulation methods may become valuable therapeutic tools in the treatment of disorders that are associated with repetitive behaviour<sup>39</sup>.

In-depth analyses of self-grooming behaviour are now an important part of behavioural phenomics (BOX 1). Several automated tools are currently available for both quantity-based and patterning-based studies of grooming phenotypes in laboratory rodents (see also REFS 95,185; Supplementary information S5 (figure)), and their future refinement is expected to contribute to progress in this field.

Given the established role of dopamine-containing neurons in movement initiation and sequencing, the regulation (and dysregulation) of the dopaminergic system in numerous brain disorders will be of particular interest for further study<sup>8,148</sup>. For example, future research may examine larger networks of molecular interactors that are related to dopaminergic genes (genes encoding proteins that directly control dopamine signalling and metabolism, cytoskeletal processes, synaptic release, Ca<sup>2+</sup>, adenosine, and glutamatergic and GABA signalling), evaluate the role of these genes in rodent self-grooming behaviour, and relate these findings to the genes that have been implicated in human brain disorders<sup>116</sup>.

Studies in rodents (including studies of rats treated with D1 agonists, mice with increased neurotransmission in the D1 circuit and DAT-deficient mice) have also shown that the activation of D1-expressing neural circuits results in the generation of excessively stereotyped, but sequentially complex, grooming patterns. This suggests that the direct output circuits of the basal ganglia are particularly important in compulsive behavioural patterns related to serial perseveration and sequential rigidity<sup>42,67–70,186</sup>. It is conceivable that basal ganglia circuitry, which is evolutionarily embedded in the control of mammalian self-grooming, could also contribute to the content of pathological human super-stereotypies. For example, in humans, mesocortico-striatal disorders that result in washing rituals or self-purification compulsions that aim to escape from perceived contamination may share similar mechanisms to self-grooming in rodents.

## Conclusions

The study of rodent self-grooming offers researchers important insights into how complex behaviours are regulated by the brain under normal circumstances and how they are affected in pathological conditions (FIG. 3). Therefore, understanding the neural circuitry, genetic determinants and associated molecular pathways that are involved in rodent self-grooming can facilitate better understanding of neurological disorders in which repetitive behaviours



are expressed. It is also possible that the brain circuitry that originally evolved to control the sequence and coordination of self-grooming as an instinctive behaviour could have been utilized throughout human evolution and cultural expansion, to extend to ritualistic behaviours, cognitive functions, and even linguistic syntax and serially ordered streams of thought<sup>123,187,188</sup>. Although this speculation remains untested, it is already clear that studies of rodent self-grooming are likely to have implications that extend beyond the motor aspects of grooming, to include the sequential control of complex behaviours in general.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Glossary

<b>Cephalocaudal progression</b>	A general direction (or rule) of rodent self-grooming behaviour that begins at the nose, then continues to the face and head, the body, the tail and the genitals.
<b>Grooming microstructure</b>	The complex sequential organization (patterning) of self-grooming movements.
<b>Fixed-action patterns</b>	Instinctive species-specific behavioural sequences that, once begun, run to their completion.
<b>Basal ganglia</b>	A group of subcortical nuclei involved in motor control, motivation and organizing movements into behavioural sequences.
<b>Ventral tegmental area</b>	A midbrain region (implicated in reward, anxiety and aversion) that contains the dopaminergic cell bodies of the mesocorticolimbic system.
<b>Research domain criteria (RDoC)</b>	A strategy in translational mental health research that aims to explore the basic mechanisms of brain deficits to understand symptom sets that are observed across multiple disorders.
<b>Behavioural perseveration</b>	The repetition of a specific behaviour that becomes inappropriate in the absence of behaviour-evoking stimuli.
<b>Stereotypies</b>	Repetitive behaviours involving an abnormal or excessive repetition of a behavioural action in the same way over time.

<b>Tics</b>	Sudden, repetitive, involuntary movements or vocalizations with varying intensity and frequency.
<b>Displacement</b>	Behaviour that is seemingly irrelevant to the context, which is displayed during a conflict of motivations or when the animal is unable to perform an activity for which it is motivated.
<b>Krabbe disease</b>	(Also known as globoid cell leukodystrophy). A rare, fatal neurodegenerative disorder that is due to genetic defect causing aberrant brain myelination.

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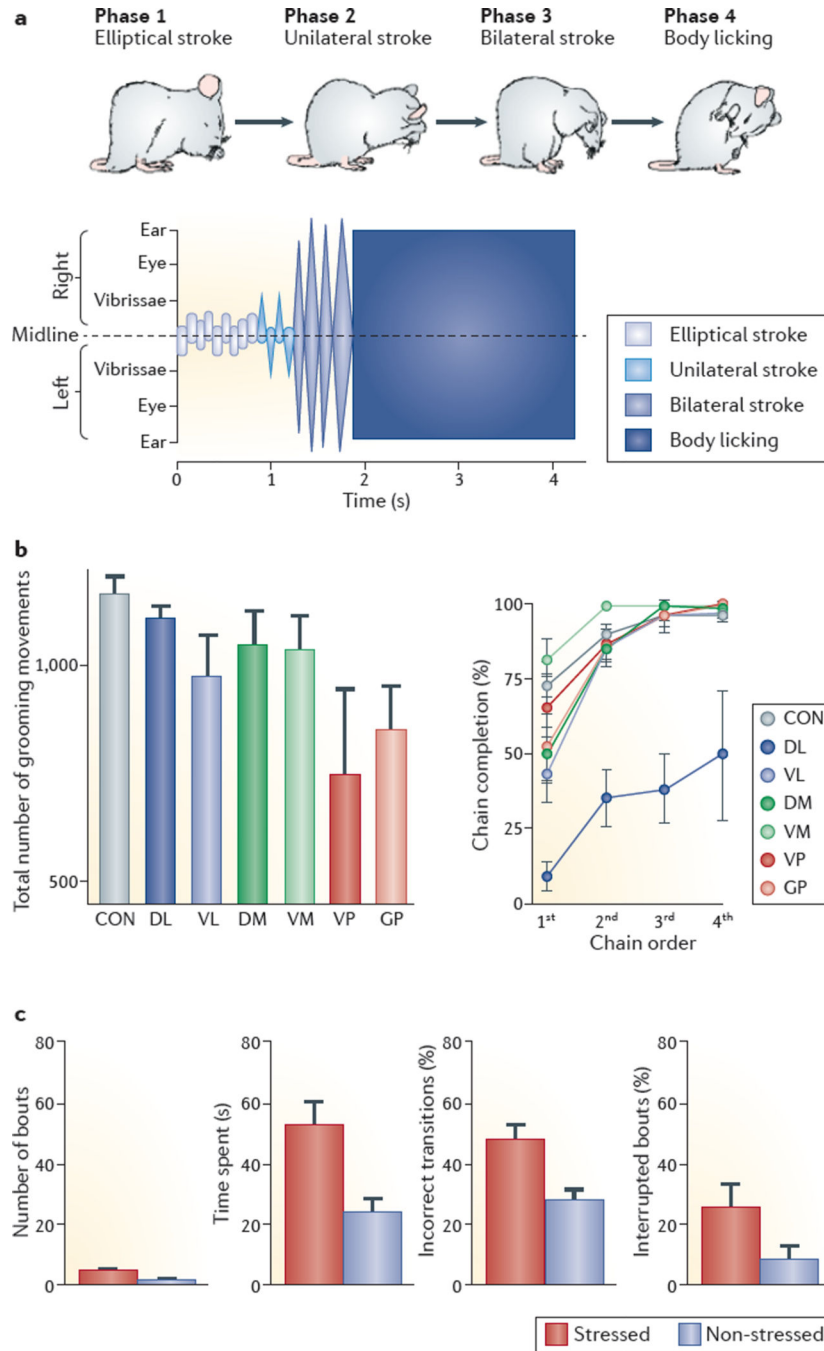
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**Box 1 | Behavioural phenomics and high-throughput analyses of grooming**

Several problems are often associated with rodent behavioural tests: they are highly time-, space- and labour-consuming, they may be expensive and they are low-medium-throughput in nature<sup>95,189</sup>. Some rodent behaviours also require a prolonged testing time to emerge, whereas others necessitate special conditions (for example, homecage testing) and/or long-term assessment to be detected. Furthermore, the behavioural response to novel drugs or genetic mutations may spontaneously appear when the animals are not being observed. These issues are particularly problematic when studying complex behaviours, such as grooming, which involves movements of multiple body points with elaborate spatiotemporal organization. However, recent advances in behavioural phenotyping have provided some timely and efficient solutions to empower grooming research. Behavioural phenomics is a rapidly developing field that merges phenomics and neuroscience, and links behavioural phenotypes to genetic and environmental factors<sup>189,190</sup>. For example, automated tools that record force, vibration or visual signals have recently been developed to allow non-invasive assessment of self-grooming that can be implemented without prior training of animals to evaluate grooming in different experimental conditions (Supplementary information S5 (figure)). Currently, such analyses cannot assess all the stages of self-grooming but can be markedly improved by using multiple cameras, three-dimensional spatial imaging of multiple body points and increased IT-based signal integration. Even more powerful tools for analysing grooming activity are likely to become available soon. For example, systems that allow the detection and integration of several different behavioural signals simultaneously (such as vibration and image) have already improved the phenotyping of rodent self-grooming<sup>95</sup>. As better signal detection and behaviour recognition capabilities continue to improve the automated analyses of grooming and affiliated behaviours, this may lead to the increased use of self-grooming analyses in high-throughput phenotyping<sup>131</sup>. A typical self-grooming patterning analysis, which previously took several days and two or three investigators to complete, can now be carried out much faster using these new technologies<sup>131,191</sup> (Supplementary information S5 (figure)).



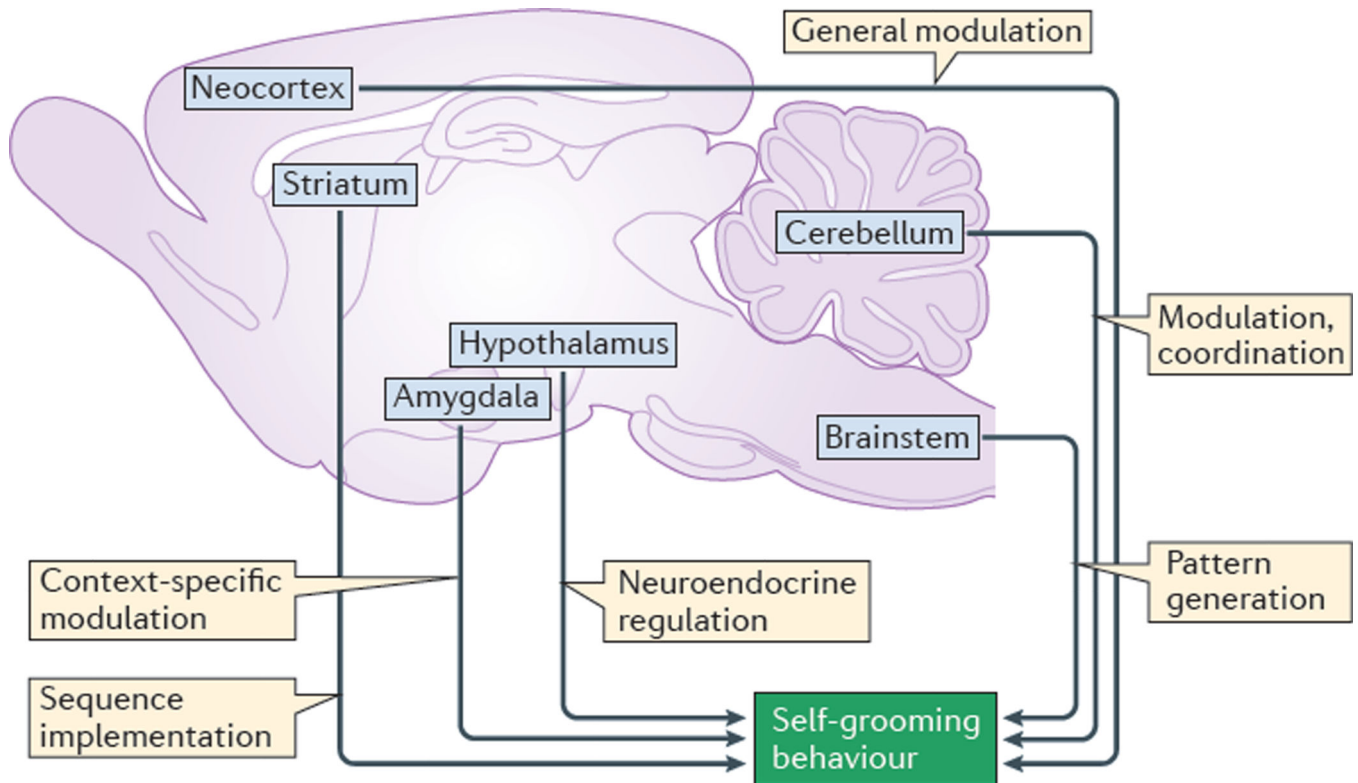


**Figure 1. Rodent self-grooming behaviour**

**a** | Mouse self-grooming has a complex sequenced structure that consists of repeated stereotyped movements known as syntactic chains<sup>9</sup> (see Supplementary information S1 and S2 (movies)). Phase 1 of a syntactic chain consists of a series of elliptical bilateral paw strokes made near the nose (paw and nose grooming); phase 2 consists of a series of unilateral strokes (each made by one paw) from the mystacial vibrissae to below the eye (face grooming); phase 3 comprises a series of bilateral strokes backwards and upwards made by both paws simultaneously (head grooming); and phase 4 consists of body licking

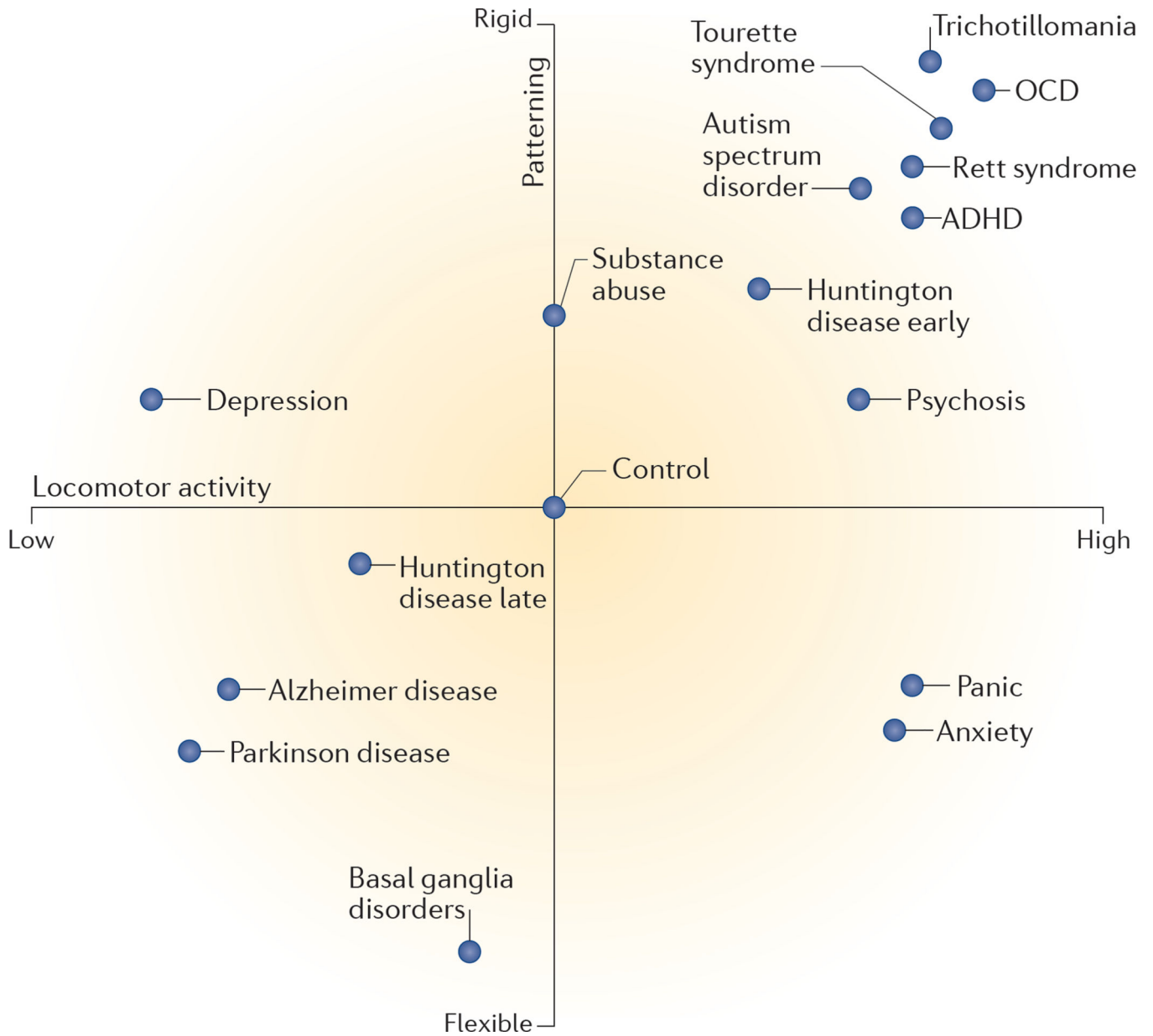


(denoted by the blue box), which is preceded by a postural cephalocaudal transition from paw–head grooming to body grooming<sup>7</sup>. In addition, grooming and scratching of the tail and genitals are frequently seen in rodents within the general cephalocaudal grooming pattern (not shown as they do not form a syntactic chain<sup>7</sup>). Although chain grooming is important for assessing the sequencing of self-grooming, note that the majority (approximately 90%) of self-grooming behaviour is represented by a more flexible, non-chain self-grooming behaviour<sup>7</sup>. **b** | Distinct localized brain lesions affect rat self-grooming behaviour in different ways<sup>34</sup>. Rats with lesions of the ventral pallidum (VP) display fewer grooming behaviours than control rats, but rats with lesions of the anterior dorsolateral striatum (DL) do not show this deficit (left). By contrast, lesions of the anterior DL result in the overt disruption of grooming sequencing (right). This is indicated by a decrease in the percentage of the first, second, third and fourth grooming chains that were begun within a session and that were completed syntactically, that is, the frequency of phases 1, 2, 3 and 4 (as illustrated in the mouse in part **a**) completed without interruption, but this parameter is not affected in rats with ventromedial striatal lesions. **c** | Example of the effects of acute stress (a 5-minute ‘aversive’ exposure to bright light) on self-grooming sequencing in rats, assessed by the ethological global analysis of chain and non-chain bouts<sup>7</sup>, including the amount of grooming activity (number of bouts and time spent grooming) and number of incorrect transitions (that is, transitions that violate the cephalocaudal rule) as a percentage of total transitions, and the number of interrupted grooming bouts as a percentage of the total. CON, control; DM, dorsomedial striatum; G P, globus pallidus; VL, ventrolateral striatum; VM, ventromedial striatum. Figure part **a** is adapted with permission from REF. 29, BMC. Figure part **b** is adapted with permission from REF. 34, Society for Neuroscience. Figure part **c** is from REF. 7, Nature Publishing Group.



**Figure 2. Brain regions involved in the regulation of rodent self-grooming**

A simplified overview of the key brain regions that are involved in different aspects of rodent self-grooming. The basal ganglia, especially the striatum and its dopaminergic inputs, control rodent self-grooming motor behaviour and its sequencing (see REF. 50 for details of the major neural connections of the basal ganglia), even for sequential patterns that are generated in the brainstem. The neocortex is involved in the general modulation of self-grooming movements, sending excitatory projections to the striatum, and receiving excitatory projections from the thalamus and amygdala. The cerebellum (including its multiple projections to the basal ganglia, thalamus, cortex, amygdala, hypothalamus, brainstem and spinal cord) is involved in motor control and coordination, and in fine-tuning of self-grooming movements. The amygdala (with its neural connections with the cortex, thalamus, hypothalamus, basal ganglia and brainstem) is involved in context-specific modulation of self-grooming (for example, during stress or in competition with social interactions)<sup>47</sup>. The hypothalamus (interconnected with the cerebellum, cortex and amygdala) is important for the neuroendocrine regulation of grooming, as stimulation of the paraventricular nucleus and the dorsal hypothalamus elicits robust self-grooming<sup>52</sup>. The hypothalamic–pituitary system also modulates self-grooming, as several hypothalamic and pituitary hormones, especially the stress-related peptides corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), potently induce rodent self-grooming<sup>56,58</sup>. Finally, the brainstem is crucial for initiating self-grooming movements and for generating basic sequential patterns (such as a syntactic chain), although by itself the brainstem is not sufficient to fully implement those patterns (as this requires striatal involvement). Figure is adapted with permission from REF. 50, *Frontiers*.



**Figure 3. Expected self-grooming behaviour in rodent models of neuropsychiatric and neurodegenerative disorders**

Aberrant self-grooming is observed in animal models of several neuropsychiatric disorders, but each disease model is expected to have distinct grooming phenotypes. Shown are the expected rodent self-grooming phenotypes relevant to particular disease models. The x-axis represents the amount of self-grooming activity that can be assessed as ranging from low frequency to high frequency, or from short to long duration, and the y-axis represents the degree of sequential patterning, ranging from rigid and repetitive to more flexible behaviour<sup>7</sup>. The expected behaviour of a wild-type control animal with normal self-grooming behaviour is shown at the centre. In this diagram, a ‘rigid’ patterning of rodent grooming, based on high adherence to the cephalocaudal progression of grooming sequence (FIG. 1), will be maximal for the stereotyped ‘chain’ grooming. By contrast, ‘flexible’

patterning denotes frequent deviations from the cephalocaudal rule, and will be maximal in 'non-chain' grooming. For example, *Sapap3*<sup>-/-</sup> mice, which display an obsessive compulsive disorder (OCD)-like phenotype, spend more time self-grooming, and their self-grooming behaviour is highly repetitive, compared with wild-type control mice<sup>40</sup>. Rodent models of anxiety (such as stress-exposed rats or mice treated with anxiogenic drugs) also display an increase in the amount of time spent grooming, but patterning of their self-grooming is impaired (see REFS 7,32 for examples). By contrast, animal models of Alzheimer disease and Parkinson disease are likely to show global progressive deficits in their grooming owing to motor impairments (for example, see REF. 160). Note that in animal models of Huntington disease, self-grooming is increased in the early stages of the animal model (see REFS 162,167 for examples), but progressive ataxia and global motor deficits (and thus decreased self-grooming) are likely to be observed at later stages, paralleling the clinical trajectory of Huntington disease. ADHD, attention deficit hyperactivity disorder.

**Table 1**

Selected rodent strains that display aberrant self-grooming behaviour

Model	Aberrant self-grooming phenotype	Refs
<i>Dat1</i> <sup>-/-</sup> mice	Increased stereotypy	29
<i>Drd1a</i> <sup>-/-</sup> mice	Increased frequency and disrupted sequencing	148
<i>Hoxb8</i> <sup>-/-</sup> mice	Excessive self-grooming	17,25
<i>Sapap3</i> <sup>-/-</sup> mice	Increased frequency and duration*	23,40,39
<i>Shank1</i> <sup>+/-</sup> and <i>Shank1</i> <sup>-/-</sup> mice	Increased duration	21
<i>Shank2</i> <sup>-/-</sup> mice	Partially increased in females (lacking exon 7) and in males (lacking exons 6 and 7)	16,112
<i>Shank3</i> <sup>+/-</sup> and <i>Shank3</i> <sup>-/-</sup> mice	Mildly increased duration	88,106,107
<i>Syn2</i> <sup>-/-</sup> mice	Increased duration	22
<i>Hdc</i> <sup>-/-</sup> mice	Increased duration	150
<i>Vdr</i> <sup>-/-</sup> mice	Increased duration and disrupted sequencing	31,158
Astrocyte-specific inducible <i>Glt1</i> <sup>-/-</sup> mice	Increased duration	85
Striatum-specific <i>Gad1</i> <sup>-/-</sup> mice	Increased duration	118
MAO-A <sup>Neo</sup> mice	Increased frequency and duration	192
BTBR mice <sup>‡</sup>	Increased duration and repetition	19,33,94
RLA rats	Increased duration	159,193,194
LY and HY rats	Different patterning in HY rats compared with LY rats	195,196

*Dat1*, dopamine transporter; *Drd1a*, dopamine receptor 1A; *Gad1*, glutamic acid decarboxylase 1; *Glt1*, excitatory amino acid transporter 2; *Hdc*, histidine decarboxylase; *Hoxb8*, homeobox protein Hox-b8; HY, selectively bred high-yawning rats; LY, selectively bred low-yawning rats; MAO-A, monoamine oxidase A; RLA, selectively bred Roman low avoidance rats; *Sapap3*, SAP90/PSD-95-associated protein 3; *Shank*, SH3 and multiple ankyrin repeat domains; *Syn2*, Synapsin II; *Vdr*, vitamin D receptor.

\* Phenotype can be reversed by genetic deletion of the gene encoding melanocortin 4 receptor, or by optogenetic stimulation of the orbitofrontal cortex and its striatal terminals.

‡ Different self-grooming activity is observed across different mouse strains used widely in research, including (from high to low in terms of self-grooming activity) DBA, C57BL/6J,129S1/SvImJ, BALB/c and NMRI, mice<sup>27,197,198</sup>.

**Table 2**

Disease symptoms that may be modelled in rodents by the assessment of self-grooming behaviour

Human disease	Symptom	Relevant rodent self- grooming phenotype	Refs
OCD	Compulsive hand washing	Increased self-grooming	37,143–145
Trichotillomania	Compulsive hair pulling	Increased self-grooming	128,199
Body dysmorphic disorder	Obsessive cosmetic grooming	Increased self-grooming	92
Excoriation	Compulsive skin-picking	Increased self-grooming	92
ASD	Behavioural perseveration	Increased self-grooming	16,19–22,33
Tourette syndrome	Tics	Increased self-grooming	29
Anxiety disorders and panic disorder	Stress-induced displacement behaviour	Increased self-grooming	7,27,31,158
Schizophrenia	Hyperarousal	Increased self-grooming	92
Trichotillomania	Compulsive hair-pulling	Increased self-barbering*	27,45
ASD	Behavioural perseveration	Grooming patterning rigidity	89–91
Depression	Behavioural perseveration	Grooming patterning rigidity	92
Anxiety disorders and panic disorder	Hyperarousal	Disrupted grooming patterning	27,28,159
Basal ganglia disorders	Impaired action sequencing	Disrupted grooming patterning	64
Depression	Anhedonia and poor hygiene	Reduced grooming activity	92
Neurodegenerative disorders	General decline in motor function	Reduced grooming activity	160

ASD, autism spectrum disorder; OCD, obsessive compulsive disorder.

\* Self-inflicted hair and whisker loss frequently seen in laboratory rodents in different contexts<sup>126</sup>. This grooming-related behaviour is an important rodent phenotype sensitive to various environmental and genetic manipulations (see Supplementary information S4 (table)).



**Table 3**

Sensitivity of rodent self-grooming to pharmacological manipulation

Model	Effect on self-grooming behaviour	Refs
<b>Chronic fluoxetine</b>		
Chronic corticostriatal stimulation in mice	Evoked grooming reversed	15
<i>Sapap3</i> <sup>-/-</sup> mice	Over-grooming and facial lesions corrected	40
<i>Slitrk5</i> <sup>-/-</sup> mice	Over-grooming and facial lesions corrected	18
<b>Chronic clomipramine</b>		
Rats selectively bred for high anxiety-like behaviour	Reduced activity	200
Rats displaying stress-evoked self-grooming	Reduced activity	157
<b>Acute memantine</b>		
Astrocyte-specific inducible <i>Glt1</i> <sup>-/-</sup> mice	Over-grooming and body lesions corrected	85
Mice prenatally exposed to valproate	Reduced over-grooming	201
<b>Acute MPEP</b>		
BTBR mouse strain	Reduced activity	90
<b>Acute risperidone</b>		
BTBR mouse strain	Reduced activity	90
<b>Acute diazepam*</b>		
Wild-type mice and rats	Reduced activity, normalized patterning during novelty-based tests	32,76
<b>Acute clonazepam*</b>		
Wild-type mice and rats	Reduced activity, normalized patterning during novelty-based tests	32,76

*Glt1*, excitatory amino acid transporter 2; MPEP, methyl-6-phenylethynyl-pyridine; *Sapap3*, SAP90/PSD-95-associated protein 3; *Slitrk5*, SLIT and NTRK-like family, member 5.

\* Benzodiazepines in general can be used as augmentation agents for nonspecific anxiolytic treatment in anti-obsessive compulsive disorder pharmacotherapy<sup>80</sup>.