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REVIEW ARTICLE

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A focus on reward in anorexia nervosa through the lens of the activity-based anorexia rodent model

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Abstract

Patients suffering anorexia nervosa (AN) become anhedonic, unable or unwilling to derive normal pleasures and tend to avoid rewarding outcomes, most profoundly in food intake. The activity-based anorexia model recapitulates many of the pathophysiological and behavioural hallmarks of the human condition, including a reduction in food intake, excessive exercise, dramatic weight loss, loss of reproductive cycles, hypothermia and anhedonia, and therefore it allows investigation into the underlying neurobiology of anorexia nervosa. The use of this model has directed attention to disruptions in central reward neurocircuitry, which may contribute to disease susceptibility. The purpose of this review is to demonstrate the utility of this unique model to provide insight into the mechanisms of reward relevant to feeding and weight loss, which may ultimately help to unravel the neurobiology of anorexia nervosa and, in a broader sense, the foundation of reward-based feeding.

KEYWORDS

activity-based anorexia, animal models, anorexia nervosa, dopamine, mesolimbic reward pathways

1 | ANOREXIA NERVOSA AS A NEUROBIOLOGICAL DISORDER

Anorexia nervosa (AN) is a debilitating psychiatric disorder of complex aetiology that is characterised by a relentless pursuit of weight loss through extreme food restriction and excessive exercise, as well as high rates of chronicity, morbidity and mortality.[1] AN is accompanied by physiological, biochemical and behavioural disturbances, including a diminished capacity to experience pleasure or reward.[2] Traditionally, AN has been considered to be caused by psychosocial factors; however, it is now clear that there are underlying neurobiological drivers.[3] Studies aiming to define the neurobiological bases of AN have relied largely on brain imaging studies of ill or recovered AN patients.[4] These have been helpful in focusing attention on the central nervous system (CNS) and suggesting the likely brain pathways and transmitters involved but, to date, have provided limited resolution of brain dysfunction or detail of underlying mechanisms. Moreover, a lack of understanding of the pathophysiology of AN has hindered the development of effective treatments, with no clear evidence available to support the efficacy of existing pharmacotherapeutic interventions beyond improving the depressive symptoms (serotonin-directed; i.e. selective serotonin reuptake inhibitors [SSRIs]) or delusional dysmorphic features (dopamine-directed; i.e. olanzapine or haloperidol) that often complicate the course of the disorder.[5] Despite this, there is considerable evidence supporting altered CNS function in AN patients, particularly for disruptions in serotonin (5-HT) and dopamine (DA) signalling. It has been proposed that 5-HT may play a role in altered satiety, impulse control and mood disruptions in AN, whereas DA may be implicated in aberrant reward and motivation related to food and feeding.[3] The main purpose of this review is to present the evidence from a rodent model of AN supporting the notion that altered central reward processing contributes to the pathogenesis of AN, with the implication that boosting the feeding reward system in AN patients may provide an effective avenue for treatment.

A prevailing neurobiological view that may underscore the extreme food restriction seen in most anorexic patients involves an imbalance between hedonic drivers of food intake on one hand and powerful inhibitory influences from the prefrontal cortex on the

other[6] (Figure 1A). A core aspect of this hypothesis is that "top-down" cognitive modulatory mechanisms exert control over "bottom-up" appetitive responses that regulate homeostatic need, responses to reward and motivational drive. The net result of these biological drivers is manifest behaviourally as high anxiety and excessive control over food intake.[7] This is supported by evidence from functional neuroimaging studies in AN that demonstrate exaggerated neural activity in the dorsolateral prefrontal cortex,[8] which leads to heightened cognitive control, and reduced neural activity in the striatum,[9] resulting in depressed reward function. These alterations combine with decreased insula activity, a region important for interoceptive awareness,[10] to create a central neural blue print for the poor recognition of homeostatic needs seen in AN patients.

2 | NEUROCIRCUITRY MODULATING REWARD AND INHIBITION

It is likely that AN behaviours are encoded in limbic and cognitive circuits that fall into two categories. The first may be described as "limbic" and involves the amygdala and ventral striatum, as well as regions of the cerebral cortex, including the insula, anterior cingulate and prefrontal regions, which collectively coordinate the affective response to stimuli including food and feeding. The current terminology introduced by Berridge and Kringelbach[11] is that "liking" is the actual pleasure component of reward and "wanting" is the measure of motivation or incentive salience for a reward. The microstructure of neural circuits and transmitters coding for pleasure can be subdivided, in simplistic terms, into (i) those dopaminergic projections originating in the ventral tegmental area (VTA) and extending to the nucleus accumbens (NAc) which account for "wanting" and (ii) the activation of μ-opioid, endocannabinoid or GABA receptors in the NAc, which enhance the affective value or hedonic characteristics of a reward such as highly palatable food.[11] The two components are recruited in the acquisition of reward, although only those in the NAc and related parts of the ventral pallidum and amygdala code for the pleasurable aspects of the reward. The other category is "cognitive" and modulates selective attention, planning and regulation of an affective state; in other words, the predisposition or willingness to undertake the affective behaviour. The regions involved in the latter are more dorsally positioned and include the dorsolateral prefrontal cortex and parietal regions. Moreover, glutamatergic circuits intrinsic to the frontal cortex extending between the orbito-frontal cortex, anterior cingulate cortex and ventromedial prefrontal cortex project to mesolimbic pathways and also influence the hedonic perception of food and other rewards[12] (Figure 1B, human; Figure 2, rodent). Brain imaging studies based in single-photon emission computed tomography, positron emission tomography (PET) and functional magnetic resonnace imaging (fMRI) analyses implicate many of these brain areas in AN and differentiate their involvement in the ill and recovered states of the condition.[4]

In addition to the interaction between these two broad determinants of anorexic behaviour, mesolimbic reward and cortical control, there are clearly modulatory influences form other regions that impinge on such circuits. One of these involves serotonin released from neurones originating in the midbrain raphe nuclei, which can influence reward processing and anhedonic behaviours via actions directly on the VTA, indirectly on the NAc (through the hippocampus) or via the ventromedial and dorsolateral prefrontal cortex, which in turn project

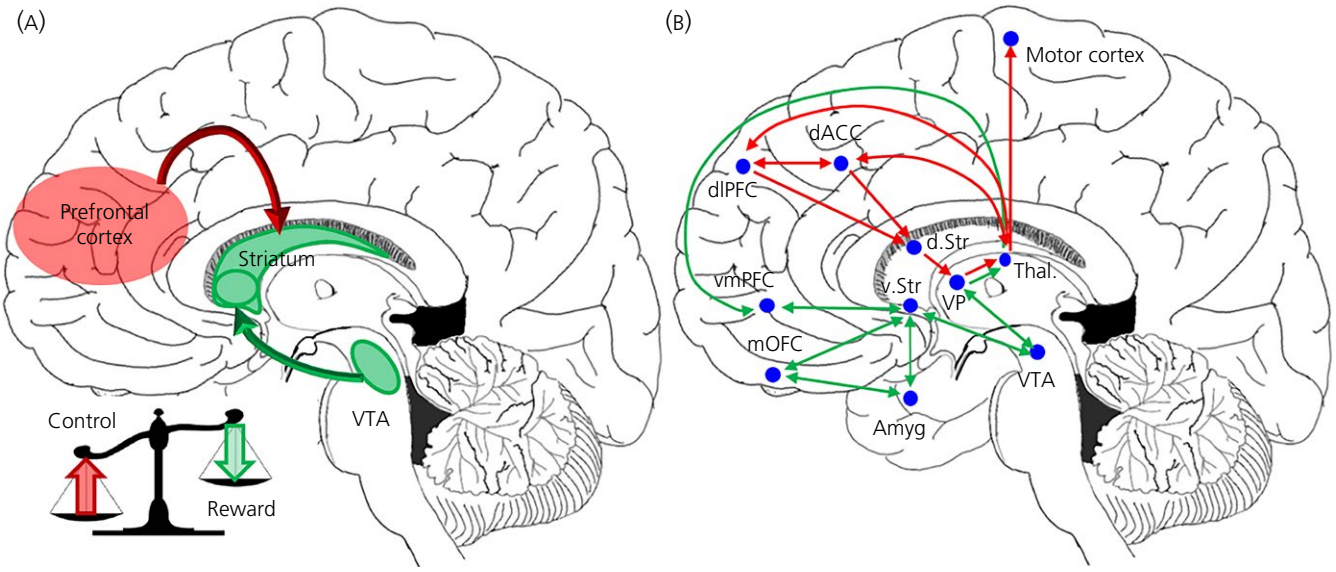

FIGURE 1 (A) An imbalance between overactive prefrontal regions in red (responsible for planning and judgment) and underactive mesolimbic regions in green (responsible for reward, emotions and conditioned effects) is proposed to underpin the neurobiology of anorexia nervosa. (B) Distinct neural pathways that contribute to executive control circuits (red arrows) and reward circuits (green arrows) have been derived from human studies. VTA, ventral tegmental area; Amyg, amygdala; VP, ventral pallidum; Thal., thalamus; v.Str, ventral striatum; d.Str, dorsal striatum; mOFC, medial orbitofrontal cortex; vmPFC, ventromedial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; dACC, dorsal anterior cingulate cortex

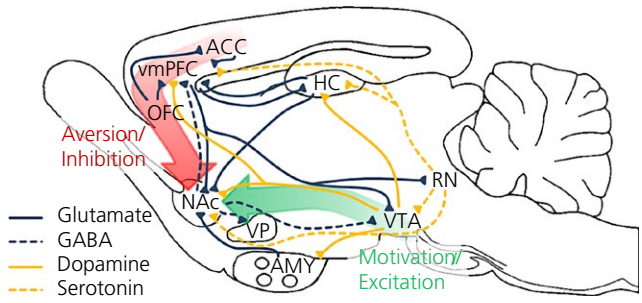

FIGURE 2 Synaptic inputs and neurotransmitter specificity of neural circuits between central reward regions, derived from studies in the rat, showing the major ventral mesolimbic pathway in green that encodes for motivated behaviours, as well as the major prefrontal cortical pathway in red that encodes for executive control, planning and inhibition. ACC, anterior cingulate cortex; AMY, amygdala; HC, hippocampus; NAc, nucleus accumbens; OFC, orbitofrontal cortex; RN, vmPFC, ventromedial prefrontal cortex; VP, ventral pallidum; VTA, ventral tegmental area

to the NAc.[12] Several lines of evidence support the notion that altered CNS serotonin function is involved in AN. First, the major metabolite of 5-HT, 5-hydroxyindoleacetic acid, is reduced in the cerebrospinal fluid of patients suffering AN and is then elevated after recovery.[13] In addition, PET studies using selective 5-HT receptor ligands demonstrate increased and decreased binding potential of cortical 5-HT_{1A} and 5-HT_{2A} receptors, respectively, in both ill and recovered AN patients,[14-18] indicative of "trait" related changes in serotonergic function that persist beyond the changing "state" of the illness. Through an action on interneurones, these receptors mediate the hyperpolarising and depolarising actions of 5-HT. The implication of these findings for the behaviour of the AN patient is a net hyperpolarisation of prefrontal cortical neurones, resulting in inhibitory or aversive attitudes.[19] There is support for this notion in the fact that there is a striking positive correlation between the binding potential of 5-HT_{1A} and 5-HT_{2A} receptors and "harm avoidance"[20,21], a behavioural trait that promotes anxiety, inhibition and inflexibility, all hallmarks of the AN condition. Although the endocannabinoid, opioid and serotonergic transmitter systems likely all contribute to generate the behavioural phenotype of AN, the recent and widespread acknowledgement that altered reward signalling and anhedonia are central to AN has directed most attention to the dopamine system.

The role of DA in reward is well researched, particularly with respect to addiction.[22] Moreover, individuals with AN have long been noted to be anhedonic and ascetic, able to sustain self-denial of not only food, but also most other comforts and pleasures in life.[3] For example, patients with AN show an increased ability to delay reward compared to healthy controls and have high punishment sensitivity and low reward reactivity during both the ill and recovered states.[23] Anhedonia has its biological underpinnings in the mesolimbic DA system[12] and it is therefore plausible that disrupted DA signalling, particularly in the mesolimbic or striatal circuitry, may contribute to altered reward-based behaviours in which there is a reduction in the incentive salience or motivation associated with the reward normally derived from food, especially highly palatable food. This view is supported by PET studies in humans that show an up-regulation of D2/D3 receptors in the ventral striatum in AN,[24,25] likely driven by a reduction in extracellular dopamine, which has been reported in the condition.[26] Gene polymorphisms in the D2 receptor have also been reported in AN patients, suggesting that DRD2 gene transcription efficiency is a susceptibility factor in the development of AN.[27] The link between anorexia and anhedonia is further supported by studies showing that patients with AN dislike highly palatable foods,[28] rate food as negative when hungry[29] and report dysphoric mood following food consumption.[30] AN patients also report more positive feelings during visual processing of underweight body images and more negative feelings for normal and overweight body images, suggesting that "thinness" could have a higher reward value for patients with AN.[31]

A substantial limitation of neurotransmitter studies in human AN is that only overall neuromodulatory effects can be measured, which precludes interrogation of the functional interactions of the many biochemical mechanisms that contribute to these complex systems. Furthermore, although disrupted function of monoamines such as dopamine and serotonin undoubtedly contribute to disease susceptibility and/or progression in AN, systemic targeting of these transmitter systems with pharmacotherapeutics has not been shown to reverse the core symptoms of the disorder. The prospect of finding targetable components of CNS circuitry that can be modulated to treat the core features of AN may be increased when altered neurotransmission is investigated within specific brain regions associated with reward function. Only with the use of valid and reliable animal models can we begin to understand the complex molecular mechanisms that drive the interactions between DA and other neurotransmitters in discrete brain regions to produce the aberrant reward processing associated with AN.

3 | THE ACTIVITY-BASED ANOREXIA MODEL

One of the challenges in studies of the CNS mechanisms underlying a range of psychiatric disorders including depression, schizophrenia and anorexia is the identification of valid animal models. Although the psychological complexity of these disorders cannot be examined in animals, such studies have furthered the development of biologically based models for disease aetiology. The most appropriate models show strong phenomenological and pathophysiological similarities and reproduce as many of the endophenotypes of the parent condition as possible. The major obstacle in relation to AN is to utilise a model that incorporates "voluntary" food restriction rather than imposed starvation; this volitional control is, for the most part, in the domain of humans. A number of animal models of AN have been proposed and trialled, including those that rely on stress, diet restriction and gene knockout (either imposed or spontaneous), although the most enduring and by far the most widely used model has been that of activity-based anorexia (ABA).[32]

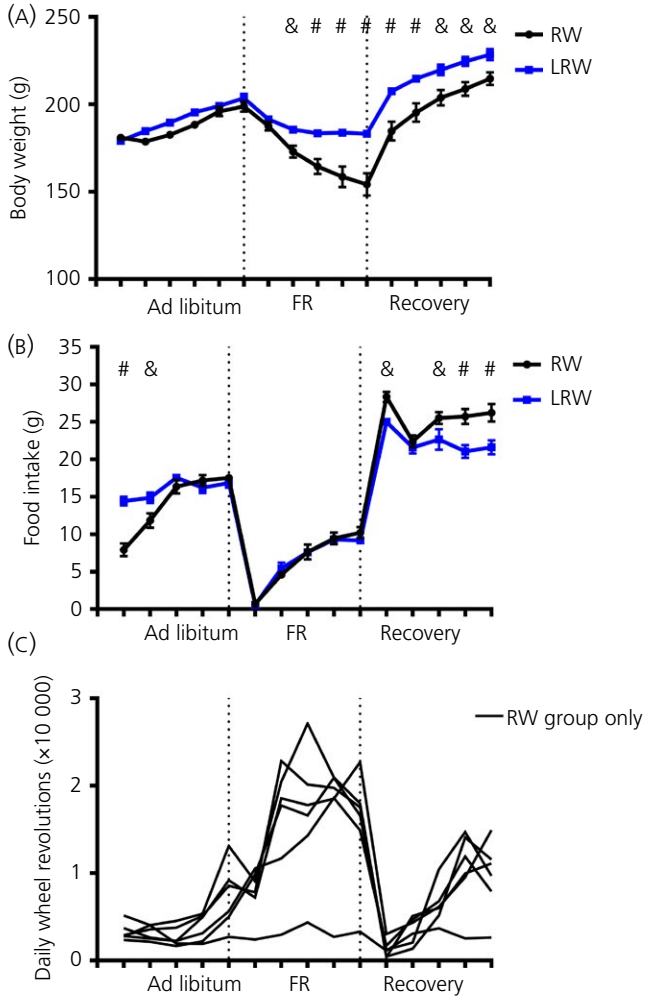

FIGURE 3 Effects of food availability on (A) body weight trajectory and (B) daily food intake for rats with locked running wheels (LRW) (n=6) or free access to running wheels (RW) (n=6) and (C) daily running wheel activity for individual RW rats. FR; Food Restriction (90 min/day). Results for (A) and (B) are expressed as mean±SEM ($^{\&}P<.01$, $^{\#}P<.001$). Reproduced with permission.[38]

Laboratory rodents are highly motivated to run in wheels and are driven to perform diverse operant responses to gain access to a wheel, although they will also engage in wheel running without any external reward whenever there is an opportunity.[33] When rats or mice with access to running wheels are placed on a restricted feeding schedule, there is a further paradoxical increase in running activity despite substantially decreased daily caloric intake, leading to a profound reduction in body weight that, without intervention, results in death by starvation.[34,35] This phenomenon was first described over 50 years ago[35] and has subsequently been referred to as ABA because it recapitulates many of the pathophysiological and behavioural hallmarks of the human condition, including a reduction in food intake, excessive exercise, dramatic weight loss, loss of reproductive cycles, hypothermia and anhedonia.[32,36,37] It is important to recognise in this paradigm that food is only limited in terms of time (usually 90 minutes at the beginning of the dark period) and not quantity, and this is why the reduction in food intake that accompanies ABA is often referred to as "voluntary". Animals quickly learn to modulate their intake within the specified time limit to maintain body weight if this is the only parameter that is changed. Similarly, given access to running wheels and unhindered access to food, they maintain their body weight quite well. It is only the combination of timed availability of food and access to running wheels that initiates the precipitous reduction in body weight that characterises the model[38,39] (Figure 3A). Under this regime, rats will typically lose 25% of their initial body weight within 1 week, and the ability to maintain body weight above this criterion of weight loss is considered as "survival" of the ABA paradigm.

Time-limited fed rats also display increased running activity in the hours preceding food presentation, known as food anticipatory activity (FAA), which has been interpreted as an evolutionary relevant foraging behaviour, useful in periods of food scarcity.[32] Mesolimbic dopamine and opioid reward circuitry underlie the reinforcing properties of running, and exert significant control on motivation and motor activity.[22] Running induced by restricted feeding (starvation-induced hyperactivity) (Figure 3C) is also a chronic stress condition that increases glucocorticoid secretion, which further modulates the function of dopamine neurones in the reward circuit.[40] Moreover, the endogenous opioids released during exercise in starved rats promote increases in pain threshold and further inhibit food intake.[41] Beyond the well-known rewarding properties of food and feeding, the consequences of running activity in ABA strengthen the case that altered reward processing is crucial to the development of the phenotype in rats, and this unique model provides a platform to investigate how reward circuit disruption may apply to human AN. Although there is general agreement that ABA is the model most analogous to human AN, as with any animal model of psychiatric disease, it has several limitations. First, rats do not experience "weight phobia", nor do they spontaneously restrict food intake, even though their food consumption is reduced compared to rats without running wheels that are subject to the same restricted feeding schedule.[32] In addition, rats will refeed immediately when ad libitum food availability is restored (Figure 3B), which is not analogous to the human AN situation. Nevertheless, it would be unrealistic to expect an animal model to reflect the entire pathology of any psychiatric disorder, and the recapitulation of the core endophenotypes by the model described above are robust and enduring.

4 | BRAIN STRUCTURE AND FUNCTION IN ABA

In addition to recapitulating the core behavioural and pathophysiological features of AN, the neurobiological changes that accompany the transition into ABA in the rodent model bear a striking resemblance to those seen in the human condition. In agreement with the central hypothesis that AN is associated with decreased ventral limbic and increased frontocortical activity, microPET analysis of cerebral ^{18}F-flurodeoxyglucose (^{18}FDG) uptake in ABA rats demonstrated lower regional metabolism in the insular cortex and ventral striatum, among other regions.[42,43] Increased ^{8}FDG metabolism in cortical regions was found to be associated with body weight loss, suggesting that this may be a substrate for starvation-induced hyperactivity.[30] Furthermore, evidence supporting the notion that changes in prefrontal cortical inhibition are central

to the reward deficits in AN has been obtained in the ABA model using electron microscopic immunocytochemistry. The lengths of axo-somatic inhibitory (GABAergic) contacts made onto layer V pyramidal cells in the prefrontal cortex of ABA mice were shown to be 45% longer than in controls,[44] indicating enhancement of inhibition in this region. Layer V is the primary excitatory output layer of the cortex; therefore, the normal cortical outflow would be blunted by enlarged inhibitory synapses in ABA, which may disrupt the control of appetitive behaviour. In this study, enlargement of inhibitory contacts in the cingulate cortex, which is linked to motor behaviours, was negatively correlated with total wheel running activity, suggesting that increased cortical inhibition prevented overall hyperactivity. Synaptic enlargement in prelimbic cortex, which is linked to cognitive and emotional behaviours, was negatively correlated with running wheel activity during the period of food access and positively correlated with FAA. Accordingly, animals with the largest inhibitory contacts ran the least during food access and had the most prominent FAA.[44] Overall cortex and corpus callosum volumes in the brains of ABA rats have also been shown to be reduced by 6% and 9%, respectively,[45] compared to controls, in line with grey and white matter reductions reported in acute AN.[46] The acute phase of ABA (ie the first 3 days) is also associated with a reduction in hippocampal cell proliferation[47] and ABA rats demonstrate alterations in dendritic branching[48] and maturation of hippocampal neurones,[49] cellular substrates that could contribute to the reduced hippocampal volumes seen in acutely ill AN patients.[50]

5 | NEUROENDOCRINE FUNCTION IN ABA

Alterations of feeding behaviour are central to the definition of AN and as such a great deal of research has been conducted into the appetite regulating centres of the brain and their biochemical modulators, including leptin and ghrelin. This circuitry based in the mediobasal hypothalamus has become one of the most widely reviewed areas of neuroendocrinology[51-53] and is not detailed here, primarily because peptidergic effects on eating behaviour in AN directly reflect reductions in body weight rather than some predisposing factor underlying disease aetiology. What is becoming clear is that key peripherally-derived regulators such as leptin and ghrelin act not only on hypothalamic and brainstem homeostatic centres controlling hunger and satiety, but also influence cognitive, emotional and rewarding components of food intake by modulating the reward neurocircuitry and neurotransmission described above.[54,55] Insights into the expanded role of hunger and satiety peptides toward reward processes stems from evidence that hypothalamic appetite-regulating circuits project to the reward-related midbrain dopamine neurocircuitry[54] and that leptin and ghrelin receptors are expressed on VTA dopaminergic neurones where they can modulate DA activity.[56-58] This highlights reciprocal connections between the appetitive and reward systems that enable integration between homeostatic and hedonic aspects of food intake.[59] The adipocyte-derived hormone leptin, which acts primarily through leptin receptors in the arcuate nucleus of the hypothalamus to decrease food intake,[51] has also been shown to modulate the activity of brain reward regions in response to food stimuli. For example, leptin treatment in patients with congenital leptin deficiency results in decreased fMRI-detected activation of hypothalamic nuclei, as well as increased activation in prefrontal cortex.[60] This suggests that the modulating effects of leptin on brain areas are not limited to midbrain-hypothalamic reward circuits but that leptin influences the more widely distributed neural network involved in reward-based feeding.

Activity-based anorexia in rats results in decreased circulating leptin and increased circulating ghrelin levels, as well as enhanced insulin sensitivity.[61] Similar to human AN, these changes reflect the marked reduction in body fat mass that occurs as animals progress through ABA and may be seen as consequences of weight loss rather than drivers of it. This may appear to be self-evident; however, efforts to reverse these neuroendocrine effects to rescue animals from the ABA phenotype have included the central or peripheral administration of a ghrelin receptor antagonist, which attenuated the ABA-associated weight loss primarily through reducing FAA.[62] Surprisingly, given the negative energy balance that is caused by ABA, leptin administration continues to decrease food intake and running wheel activity in ABA,[63] suggesting that a hypersensitivity to the effects of leptin for ABA rats may be linked to the overlap between leptin and DA receptors in reward circuits.[56] In addition, the melanocortin system has been implicated in ABA, with conflicting results. Here, the precursor peptide pro-opiomelanocortin (POMC) is down-regulated after rats are subjected to 7 days of the ABA paradigm.[64] However, chronic treatment with α-melanocyte-stimulating hormone, a peptide cleaved from POMC, exacerbates the ABA phenotype by increasing wheel running and decreasing food intake.[65] In addition, suppression of the melanocortin system with central agouti-related peptide administration ameliorates the ABA phenotype,[66] even though ABA is associated with an upregulation of agouti-related peptide.[64] It would appear then that there has not been a clear indication of the role of peptide transmitters involved in energy homeostasis in the aetiology of ABA, and similarly of AN.

Because neuroendocrine abnormalities appear to be associated only with the acute phase of the disorder in humans, and generally disappear with recovery, it is likely, as noted above, that these changes are a consequence of body weight loss rather than an aetiological or predisposing factor. It is therefore questionable whether studies utilising ABA to uncover changes in such peptides will contribute to an understanding of the genesis of AN. Furthermore, patients with AN show decreased sensitivity to the motivational drive of hunger,[67] and so modification of hunger or satiety factors is not likely to represent a viable treatment option for the disorder. Conversely, it is important to remember that there is a well established role for feeding regulators in the modulation of reward[56] and, although such peptides may not be the essential drivers of the AN condition, there is little doubt that altered endocrine function contributes to the overall disruption of reward signalling in AN.[55]

6 | SEROTONIN DISRUPTIONS IN ABA

Although a large body of research on anorexia has focused on serotonergic systems, therapeutic interventions targeting 5-HT have had

little success in treating the core symptoms of the disorder[5] and may be more relevant to associated mood disturbances. 5-HT is known to have a suppressive effect on food intake,[68] and so it appears paradoxical that increasing the availability of 5-HT with the use of SSRIs might promote survival of ABA. Indeed, mice treated with the SSRI fluoxetine during the ABA paradigm showed increased food intake and reduced FAA; however, survival of the ABA paradigm was not increased compared to vehicle-treated mice.[69] Furthermore, treatment with d-fenfluramine, a drug that stimulates 5-HT release and blocks its reuptake, primarily through the 5-HT_{2C} receptor, either did not impact the ABA phenotype[70] or accelerated the associated weight loss[71] and selective 5-HT receptor antagonism promoted weight loss in ABA.[72] Conversely, decreasing 5-HT signalling by treatment with the 5-HT antagonist 8-OHDPAT was shown to prevent starvation-induced hyperactivity in ABA without changing food intake.[73] Despite these unconvincing findings, it is important to note that the 5-HT system is particularly complex, with 14 (or more) receptor subtypes and many other components that modulate regionally-specific effects.[3] Thus, it is likely is that serotonergic disruptions in AN and ABA will be specific to regions of the brain involved in reward processing, such that the type of global increase or decrease in available 5-HT would be unable to capture these changes for effective treatment.

Recently, some traction has been gained in the involvement of 5-HT signalling on food intake within brain regions relevant to reward processing. Directly infusing agonists and antagonists directed at different 5-HT receptor subtypes into the VTA showed subtype-specific effects on feeding behaviour in food restricted rats. For example, 5-HT_{1A} and 5-HT_{1B} agonism inhibited chow food intake whereas 5-HT_{2B} stimulation did not affect chow intake but significantly increased intake of highly palatable food,[74] indicating that the effects of serotonin on reward-based feeding depends on the receptor subtype targeted and the reward context of the food. These effects are also most likely to be specific to brain regions involved in reward and, indeed, levels of 5-HT and its metabolites specifically within the NAc were shown to be maintained at a lower level in animals undergoing ABA compared to control levels.[75] Considering the likely involvement of 5-HT in psychological and mood disruptions in AN, the behavioural component of ABA may not be the most appropriate model for determining the underlying mechanisms of 5-HT disruption. In light of the evidence suggesting that specific 5-HT receptor subtypes in VTA are associated with specific aspects of food intake,[74] the question of how 5-HT signalling is involved in reward and AN may be better suited to investigation with cellular pharmacology techniques in combination with patch-clamp electrophysiology.

7 | DOPAMINE AND REWARD DISRUPTIONS IN ABA

More promising steps toward uncovering a target for treatment development have been through investigations of specific dopamine-related actions associated with different aspects of the ABA phenotype. From the inception of the model, attempts to prevent or rescue animals from ABA have focused attention on DA signalling. One aspect of this relates to the fact that chlorpromazine reduces activity and increases food intake during ABA.[35] More recently, excessive running activity in ABA rats was ameliorated with a nonselective dopamine antagonist *cis*-flupentixol, which increased food intake in ABA but not ad libitum fed rats, thus preventing body weight loss.[76] It is important to note that FAA persisted in treated ABA rats, which argues against the involvement of dopamine in anticipatory activity, even though FAA decreases after dopaminergic antagonism in ad libitum fed mice.[77] It may be that the behavioural responses to global DA antagonism differ in the altered reward context of the ABA paradigm. Selective antagonism of DA2/3 receptors is shown to ameliorate ABA by increasing food intake to a greater extent than olanzapine administration, which is an antagonist to 5-HT2 and DA receptors D1-5[72], suggesting that the effects of D2/3 may underlie the mechanism of action of olanzapine in reducing ABA symptoms.[69,78] Because dopamine is integral to mesolimbic reward processing, it follows logically that central reward dysfunction in AN and ABA is associated with the DA disturbances described above, specifically within the mesolimbic reward circuit. Indeed, rodent studies indicate that the ABA phenotype can be ameliorated by increasing the hedonic or rewarding value of food, by substituting laboratory chow with a high-fat diet alone[79]

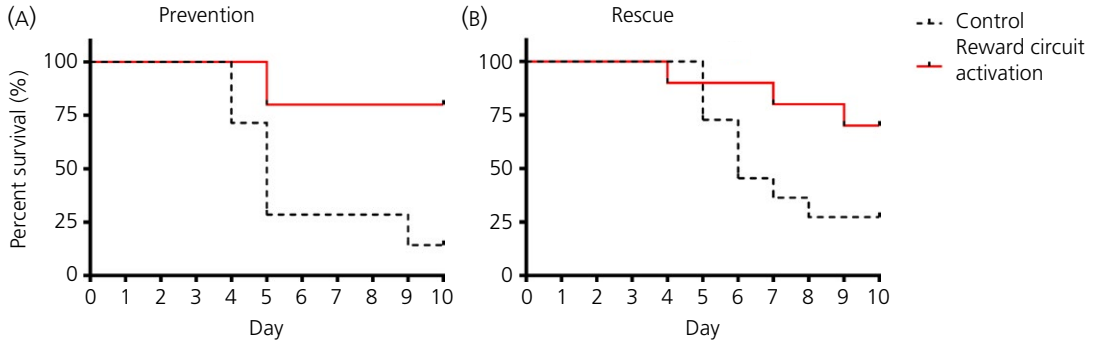

FIGURE 4 Survival curves for DREADD activation of the reward circuit in the ventral tegmental area, extending to the nucleus accumbens, during activity-based anorexia (ABA). Activation of the reward circuitry promoted survival during prevention of the ABA phenotype (A) in which 80% vs 14% survived [χ^2=9.95, d.f.=1, *P*=.002] as well as during rescue experiments (B) in which 75% vs 25% survived [χ^2=3.97, d.f.=1, *P*=.046)]. Produced from data in Foldi et al.[38]

or combined with delta-9-thetrahydrocannabinol (THC) administration,[37] both of which have effects mediated by DA signalling.[80]

The DA-mediated changes in reward circuits are now being disentangled by precise and specialised interrogation of the ABA model. For example, using microdialysis in animals exposed to the ABA paradigm, hourly samples of DA release have been taken and measured to assess the dynamic changes in DA and 5-HT and their metabolites during ABA, specifically within the NAc, followed by correlation with ABA-associated behaviours. This targeted approach revealed that DA levels increased during food intake but not FAA. It also demonstrated that ABA rats maintained lower 5-HT levels in NAc than ad libitum fed rats, although this was not correlated with behaviour.[75] Further studies of this nature will allow insight into the complex interplay of 5HT and DA in the development of ABA, interrogating the hypothesis that 5-HT signalling as a key substrate for the aversive motivational system interacting with the DA-related appetitive system. Recent advances in technology in the neurosciences have provided an unprecedented ability to manipulate, both temporally and spatially and using optogenetic and chemogenetic technologies in combination with behavioural and metabolic techniques, the function of specific neural relays that are involved in ABA. Our recently published data demonstrate that the ABA phenotype can be prevented by direct activation of the VTA-NAc reward pathway, using a dual viral strategy to identify and manipulate pathway-specific projections. Briefly, this approach involves stereotaxically injecting canine adenovirus (CAV), expressing Cre recombinase (CAV-2-Cre) into the NAc, which is retrogradely transported to the VTA and recombines with the separately injected activating DREADD virus tagged with a fluorescent reporter [AAV-hSyn-DIO-$hM_3D(G_q)$-mCherry], resulting in activation of this neuroanatomically defined pathway. This strategy revealed that the drastic body weight "free-fall" associated with ABA can be prevented and rescued by reward circuit activation, leading to an increase in food intake and FAA, with a profound increase in survival (Figure 4).[38] The continuation and extension of these targeted studies will directly inform questions about the necessity and sufficiency of the circuitry underpinning ABA and, potentially, the neurobiology of AN.

8 | TAKING THE ABA MODEL INTO THE FUTURE

This review has demonstrated the utility of the ABA model in highlighting the role of aberrant reward processing in the development and maintenance of the ABA phenotype and, moreover, in furthering the notion that treatment opportunities for patients with AN may include enhancing the reward associated with feeding. We predict that the future of research into reward neurocircuitry in the ABA model will mirror the future of the technical landscape of research in the neurosciences. For example, chemogenetic and optogenetic techniques now allow us to enhance or depress neuronal activity across cellular, circuit-level or brain-wide spatial scales and will provide powerful tests of the native, necessary and sufficient causal underpinnings of the physiological and behavioural phenotype of ABA. Studies have already begun using these technologies to show dissociable pathways relevant to AN behaviours, whereby activation of dopamine neurones in the VTA but not the substantia nigra (SN) induced a pronounced and long-lasting hyperactive phenotype,[81] whereas activation of both VTA and SN dopamine neurones impaired attention in a behavioural assay known as the five choice serial reaction time task.[82] Taking the ABA model into the future by exploiting it in combination with novel neuroscience techniques and technologies will enable us to answer specific questions about the involvement of brain regions and transmitters in behavioural features of ABA.

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: APA; 2013.
2. Klein DA, Walsh BT. Eating disorders: clinical features and pathophysiology. *Physiol Behav*. 2004;81:359-374.
3. Kaye WH, Wierenga CE, Bailer UF, Simmons AN, Bischoff-Grethe A. Nothing tastes as good as skinny feels: the neurobiology of anorexia nervosa. *Trends Neurosci*. 2013;36:110-120.
4. van Kuyck K, Gerard N, Van Laere K, et al. Towards a neurocircuitry in anorexia nervosa: evidence from functional neuroimaging studies. *J Psychiatr Res*. 2009;43:1133-1145.
5. Miniati M, Mauri M, Ciberti A, Mariani MG, Marazziti D, Dell'Osso L. Psychopharmacological options for adult patients with anorexia nervosa. *CNS Spectr*. 2016;21:134-142.
6. Wierenga CE, Ely A, Bischoff-Grethe A, Bailer UF, Simmons AN, Kaye WH. Are extremes of consumption in eating disorders related to an altered balance between reward and inhibition? *Front Behav Neurosci*. 2014;8:410.
7. O'Hara CB, Campbell IC, Schmidt U. A reward-centred model of anorexia nervosa: a focussed narrative review of the neurological and psychophysiological literature. *Neurosci Biobehav Rev*. 2015;52:131-152.
8. Ehrlich S, Geisler D, Ritschel F, et al. Elevated cognitive control over reward processing in recovered female patients with anorexia nervosa. *J Psychiatry Neurosci*. 2015;40:140249.
9. Wagner A, Aizenstein H, Mazurkewicz L, et al. Altered insula response to taste stimuli in individuals recovered from restricting-type anorexia nervosa. *Neuropsychopharmacology*. 2008;33:513-523.
10. Frank GK, Collier S, Shott ME, O'Reilly RC. Prediction error and somatosensory insula activation in women recovered from anorexia nervosa. *J Psychiatry Neurosci*. 2016;41:304-311.
11. Berridge KC, Kringelbach ML. Affective neuroscience of pleasure: reward in humans and animals. *Psychopharmacology*. 2008;199:457-480.
12. Der-Avakian A, Markou A. The neurobiology of anhedonia and other reward-related deficits. *Trends Neurosci*. 2012;35:68-77.
13. Stanley M, Traskman-Bendz L, Dorovini-Zis K. Correlations between aminergic metabolites simultaneously obtained from human CSF and brain. *Life Sci*. 1985;37:1279-1286.
14. Frank GK, Kaye WH, Meltzer CC, et al. Reduced 5-HT2A receptor binding after recovery from anorexia nervosa. *Biol Psychiatry*. 2002;52:896-906.
15. Audenaert K, Van Laere K, Dumont F, et al. Decreased 5-HT2a receptor binding in patients with anorexia nervosa. *J Nucl Med*. 2003;44:163-169.
16. Bailer UF, Frank GK, Henry SE, et al. Exaggerated 5-HT1A but normal 5-HT2A receptor activity in individuals ill with anorexia nervosa. *Biol Psychiatry*. 2007;61:1090-1099.
17. Bailer UF, Frank GK, Henry SE, et al. Altered brain serotonin 5-HT1A receptor binding after recovery from anorexia nervosa measured by positron emission tomography and [carbonyl11C] WAY-100635. *Arch Gen Psychiatry*. 2005;62:1032-1041.

18. Galusca B, Costes N, Zito NG, et al. Organic background of restrictive-type anorexia nervosa suggested by increased serotonin 1A receptor binding in right frontotemporal cortex of both lean and recovered patients: [^{18}F] MPPF PET scan study. *Biol Psychiatry*. 2008;64:1009-1013.
19. Amargos-Bosch M, Bortolozzi A, Puig MV, et al. Co-expression and in vivo interaction of serotonin1A and serotonin2A receptors in pyramidal neurons of prefrontal cortex. *Cereb Cortex*. 2004;14:281-299.
20. Weisstaub NV, Zhou M, Lira A, et al. Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. *Science*. 2006;313:536-540.
21. Moresco FM, Dieci M, Vita A, et al. In vivo serotonin 5HT(2A) receptor binding and personality traits in healthy subjects: a positron emission tomography study. *NeuroImage*. 2002;17:1470-1478.
22. Wise RA. Dopamine, learning and motivation. *Nat Rev Neurosci*. 2004;5:483-494.
23. Harrison A, O'Brien N, Lopez C, Treasure J. Sensitivity to reward and punishment in eating disorders. *Psychiatry Res*. 2010;177:1-11.
24. Bailer UF, Frank GK, Price JC, et al. Interaction between serotonin transporter and dopamine D2/D3 receptor radioligand measures is associated with harm avoidant symptoms in anorexia and bulimia nervosa. *Psychiatry Res*. 2013;211:160-168.
25. Frank GK, Bailer UF, Henry SE, et al. Increased dopamine D2/D3 receptor binding after recovery from anorexia nervosa measured by positron emission tomography and [11c] raclopride. *Biol Psychiatry*. 2005;58:908-912.
26. Kaye WH, Frank GK, McConaha C. Altered dopamine activity after recovery from restricting-type anorexia nervosa. *Neuropsychopharmacology*. 1999;21:503-506.
27. Bergen AW, Yeager M, Welch RA, et al. Association of multiple DRD2 polymorphisms with anorexia nervosa. *Neuropsychopharmacology*. 2005;30:1703-1710.
28. Fernstrom MH, Weltzin TE, Neuberger S, Srinivasagam N, Kaye WH. Twenty-four-hour food intake in patients with anorexia nervosa and in healthy control subjects. *Biol Psychiatry*. 1994;36:696-702.
29. Santel S, Baving L, Krauel K, Munte TF, Rotte M. Hunger and satiety in anorexia nervosa: fMRI during cognitive processing of food pictures. *Brain Res*. 2006;1114:138-148.
30. Kaye W. Neurobiology of anorexia and bulimia nervosa. *Physiol Behav*. 2008;94:121-135.
31. Clarke J, Ramoz N, Fladung AK, Gorwood P. Higher reward value of starvation imagery in anorexia nervosa and association with the Val66Met BDNF polymorphism. *Transl Psychiatry*. 2016;6:e829.
32. Gutierrez E. A rat in the labyrinth of anorexia nervosa: contributions of the activity-based anorexia rodent model to the understanding of anorexia nervosa. *Int J Eat Disord*. 2013;46:289-301.
33. Sherwin CM. Voluntary wheel running: a review and novel interpretation. *Anim Behav*. 1998;56:11-27.
34. Epling FW, Pierce DW, Stefan L. A theory of activity-based anorexia. *Int J Eat Disord*. 1983;3:27-46.
35. Routtenberg A, Kuznesof AW. Self-starvation of rats living in activity wheels on a restricted feeding schedule. *J Comp Physiol Psychol*. 1967;64:414-421.
36. Dixon DP, Ackert AM, Eckel LA. Development of, and recovery from, activity-based anorexia in female rats. *Physiol Behav*. 2003;80:273-279.
37. Verty AN, Evetts MJ, Crouch GJ, McGregor IS, Stefanidis A, Oldfield BJ. The cannabinoid receptor agonist THC attenuates weight loss in a rodent model of activity-based anorexia. *Neuropsychopharmacology*. 2011;36:1349-1358.
38. Foldi CJ, Milton LK, Oldfield BJ. The role of mesolimbic reward neurocircuitry in prevention and rescue of the activity-based anorexia (ABA) phenotype in rats. *Neuropsychopharmacology*. 2017. https://doi.org/10.1038/npp.2017.63. [Epub ahead of print].
39. Scharner S, Prinz P, Goebel-Stengel M, et al. Activity-based anorexia reduces body weight without inducing a separate food intake microstructure or activity phenotype in female rats-mediation via an activation of distinct brain nuclei. *Front Neurosci*. 2016;10:475.
40. Burden VR, White BD, Dean RG, Martin RJ. Activity of the hypothalamic-pituitary-adrenal axis is elevated in rats with activity-based anorexia. *J Nutr*. 1993;123:1217-1225.
41. Aravich PF, Rieg TS, Lauterio TJ, Doerries LE. Beta-endorphin and dynorphin abnormalities in rats subjected to exercise and restricted feeding: relationship to anorexia nervosa? *Brain Res*. 1993;622:1-8.
42. van Kuyck K, Casteels C, Vermaelen P, Bormans G, Nuttin B, Van Laere K. Motor- and food-related metabolic cerebral changes in the activity-based rat model for anorexia nervosa: a voxel-based micro-PET study. *NeuroImage*. 2007;35:214-221.
43. Barbarich-Marsteller NC, Marsteller DA, Alexoff DL, Fowler JS, Dewey SL. MicroPET imaging in an animal model of anorexia nervosa. *Synapse (New York, NY)*. 2005;57:85-90.
44. Chen YW, Wable GS, Chowdhury TG, Aoki C. Enlargement of axo-somatic contacts formed by GAD-immunoreactive axon terminals onto layer V pyramidal neurons in the medial prefrontal cortex of adolescent female mice is associated with suppression of food restriction-evoked hyperactivity and resilience to activity-based anorexia. *Cereb Cortex*. 2016;26:2574-2589.
45. Frintrop L, Liesbrock J, Paulukat L, et al. Reduced astrocyte density underlying brain volume reduction in activity-based anorexia rats. *World J Biol Psychiatry*. 2017; doi: 10.1080/15622975.2016.1273552. [Epub ahead of print]:1-11.
46. Seitz J, Herpertz-Dahlmann B, Konrad K. Brain morphological changes in adolescent and adult patients with anorexia nervosa. *J Neural Transm (Vienna, Austria: 1996)*. 2016;123:949-959.
47. Barbarich-Marsteller NC, Fornal CA, Takase LF, et al. Activity-based anorexia is associated with reduced hippocampal cell proliferation in adolescent female rats. *Behav Brain Res*. 2013;236:251-257.
48. Chowdhury TG, Barbarich-Marsteller NC, Chan TE, Aoki C. Activity-based anorexia has differential effects on apical dendritic branching in dorsal and ventral hippocampal CA1. *Brain Struct Funct*. 2014;219:1935-1945.
49. Chowdhury TG, Rios MB, Chan TE, Cassataro DS, Barbarich-Marsteller NC, Aoki C. Activity-based anorexia during adolescence disrupts normal development of the CA1 pyramidal cells in the ventral hippocampus of female rats. *Hippocampus*. 2014;24:1421-1429.
50. Connan F, Murphy F, Connor SE, et al. Hippocampal volume and cognitive function in anorexia nervosa. *Psychiatry Res*. 2006;146:117-125.
51. Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron*. 1999;22:221-232.
52. Zha X, Xu X. Dissecting the hypothalamic pathways that underlie innate behaviors. *Neurosci Bull*. 2015;31:629-648.
53. Sainsbury A, Zhang L. Role of the hypothalamus in the neuroendocrine regulation of body weight and composition during energy deficit. *Obes Rev*. 2012;13:234-257.
54. Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci*. 2011;15:37-46.
55. Monteleone AM, Castellini G, Volpe U, et al. Neuroendocrinology and brain imaging of reward in eating disorders: a possible key to the treatment of anorexia nervosa and bulimia nervosa. *Prog Neuropsychopharmacol Biol Psychiatry*. 2017; pii: S0278-5846(16)30488-2. doi: 10.1016/j.pnpbp.2017.02.020. [Epub ahead of print].
56. Hommel JD, Trinko R, Sears RM, et al. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron*. 2006;51:801-810.
57. Abizaid A, Liu ZW, Andrews ZB, et al. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest*. 2006;116:3229-3239.
58. Krugel U, Schraft T, Kittner H, Kiess W, Illes P. Basal and feeding-evoked dopamine release in the rat nucleus accumbens is depressed by leptin. *Eur J Pharmacol*. 2003;482:185-187.

59. Berthoud HR, Munzberg H, Morrison CD. Blaming the brain for obesity: integration of hedonic and homeostatic mechanisms. *Gastroenterology*. 2017;152:1728-1738.
60. Baicy K, London ED, Monterosso J, et al. Leptin replacement alters brain response to food cues in genetically leptin-deficient adults. *Proc Natl Acad Sci USA*. 2007;104:18276-18279.
61. Pardo M, Roca-Rivada A, Al-Massadi O, Seoane LM, Camina JP, Casanueva FF. Peripheral leptin and ghrelin receptors are regulated in a tissue-specific manner in activity-based anorexia. *Peptides*. 2010;31:1912-1919.
62. Verhagen LA, Egecioglu E, Luijendijk MC, Hillebrand JJ, Adan RA, Dickson SL. Acute and chronic suppression of the central ghrelin signaling system reveals a role in food anticipatory activity. *Eur Neuropsychopharmacol*. 2011;21:384-392.
63. Hillebrand JJ, Koeners MP, de Rijke CE, Kas MJ, Adan RA. Leptin treatment in activity-based anorexia. *Biol Psychiatry*. 2005;58:165-171.
64. Adan RA, Hillebrand JJ, De Rijke C, et al. Melanocortin system and eating disorders. *Ann NY Acad Sci*. 2003;994:267-274.
65. Hillebrand JJ, Kas MJ, Adan RA. alpha-MSH enhances activity-based anorexia. *Peptides*. 2005;26:1690-1696.
66. Kas MJ, van Dijk G, Scheurink AJ, Adan RA. Agouti-related protein prevents self-starvation. *Mol Psychiatry*. 2003;8:235-240.
67. Wierenga CE, Bischoff-Grethe A, Melrose AJ, et al. Hunger does not motivate reward in women remitted from anorexia nervosa. *Biol Psychiatry*. 2015;77:642-652.
68. Simansky KJ. Serotonergic control of the organization of feeding and satiety. *Behav Brain Res*. 1996;73:37-42.
69. Klenotich SJ, Seiglie MP, McMurray MS, et al. Olanzapine, but not fluoxetine, treatment increases survival in activity-based anorexia in mice. *Neuropsychopharmacology*. 2012;37:1620-1631.
70. Hillebrand JJ, Heinsbroek AC, Kas MJ, Adan RA. The appetite suppressant d-fenfluramine reduces water intake, but not food intake, in activity-based anorexia. *J Mol Endocrinol*. 2006;36:153-162.
71. Atchley DP, Eckel LA. Fenfluramine treatment in female rats accelerates the weight loss associated with activity-based anorexia. *Pharmacol Biochem Behav*. 2005;80:273-279.
72. Klenotich SJ, Ho EV, McMurray MS, Server CH, Dulawa SC. Dopamine D2/3 receptor antagonism reduces activity-based anorexia. *Transl Psychiatry*. 2015;5:e613.
73. Atchley DP, Eckel LA. Treatment with 8-OH-DPAT attenuates the weight loss associated with activity-based anorexia in female rats. *Pharmacol Biochem Behav*. 2006;83:547-553.
74. Pratt WE, Clissold KA, Lin P, et al. A systematic investigation of the differential roles for ventral tegmentum serotonin 1- and 2-type receptors on food intake in the rat. *Brain Res*. 2016;1648(Pt A):54-68.
75. Verhagen LA, Luijendijk MC, Korte-Bouws GA, Korte SM, Adan RA. Dopamine and serotonin release in the nucleus accumbens during starvation-induced hyperactivity. *Eur Neuropsychopharmacol*. 2009;19:309-316.
76. Verhagen LA, Luijendijk MC, Hillebrand JJ, Adan RA. Dopamine antagonism inhibits anorectic behavior in an animal model for anorexia nervosa. *Eur Neuropsychopharmacol*. 2009;19:153-160.
77. Liu YY, Liu TY, Qu WM, Hong ZY, Urade Y, Huang ZL. Dopamine is involved in food-anticipatory activity in mice. *J Biol Rhythms*. 2012;27:398-409.
78. Hillebrand JJ, van Elburg AA, Kas MJ, van Engeland H, Adan RA. Olanzapine reduces physical activity in rats exposed to activity-based anorexia: possible implications for treatment of anorexia nervosa? *Biol Psychiatry*. 2005;58:651-657.
79. Brown AJ, Avena NM, Hoebel BG. A high-fat diet prevents and reverses the development of activity-based anorexia in rats. *Int J Eat Disord*. 2008;41:383-389.
80. Malone DT, Taylor DA. Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. *Br J Pharmacol*. 1999;128:21-26.
81. Boekhoudt L, Omrani A, Luijendijk MC, et al. Chemogenetic activation of dopamine neurons in the ventral tegmental area, but not substantia nigra, induces hyperactivity in rats. *Eur Neuropsychopharmacol*. 2016;26:1784-1793.
82. Boekhoudt L, Voets ES, Flores-Dourojeanni JP, Luijendijk MC, Vanderschuren LJ, Adan RA. Chemogenetic activation of midbrain dopamine neurons affects attention, but not impulsivity, in the five-choice serial reaction time task in rats. *Neuropsychopharmacology*. 2016;42:1315-1325.

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