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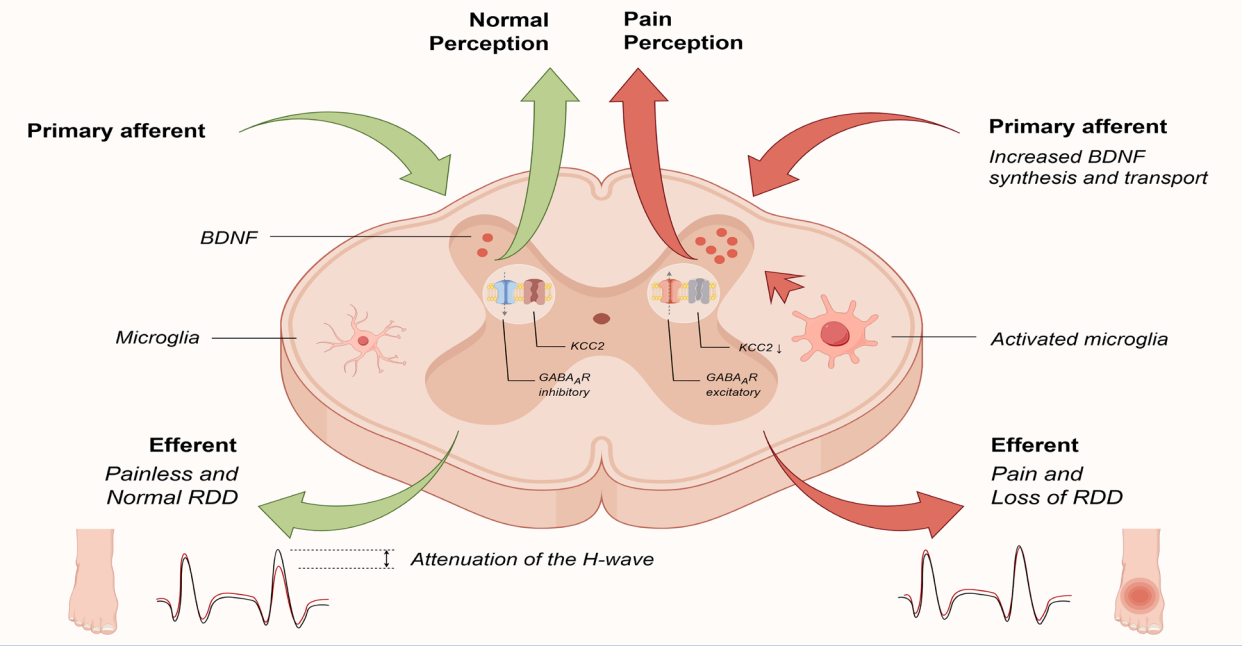

BDNF: Brain-derived neurotrophic factor; RDD: Rate-dependent depression

Highlights

- The H-reflex offers insight into both reflex integrity and synaptic regulation.
- RDD, a modified H-reflex technique, is widely used to assess movement disorders.
- RDD is impaired in rodents and some humans with painful DPN.
- Impaired RDD may serve as a biomarker for spinally-mediated pain in DPN.
- Using RDD in painful DPN could aid in tailoring personalized analgesic strategies.

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Rate-Dependent Depression of the Hoffmann Reflex: Practical Applications in Painful Diabetic Neuropathy

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Measurement of the rate-dependent depression (RDD) of the Hoffmann (H) reflex, a technique developed over half a century ago, is founded on repeated stimulation of the H-reflex with tracking of sequentially evoked H-wave amplitudes in the resulting electromyogram. RDD offers insight into the integrity of spinal reflex pathways and spinal inhibitory regulation. Initially, RDD was predominantly utilized in the mechanistic exploration and evaluation of movement disorders characterized by spasticity symptoms, as may occur following spinal cord injury. However, there is increasing recognition that sensory input from the periphery is modified at the spinal level before ascending to the higher central nervous system and that some pain states can arise from, or be exaggerated by, disruption of spinal processing via a mechanism termed spinal disinhibition. This, along with the urgent clinical need to identify biological markers of pain generator and/or amplifier sites to facilitate targeted pain therapies, has prompted interest in RDD as a biomarker for the contribution of spinal disinhibition to neuropathic pain states. Current research in animals and humans with diabetes has revealed specific disorders of spinal GABAergic function associated with impaired RDD. Future investigations on RDD aim to further elucidate its underlying pathways and enhance its clinical applications.

Keywords: Diabetic neuropathies; Electrophysiology; H-reflex; Neuralgia; Neural inhibition

INTRODUCTION

The spinal reflex plays a pivotal role in motor coordination and sensory perception and thus can be used to assess physiological or pathological changes within the nervous system. While the manual percussion hammer remains a valuable tool, electrophysiological techniques offer quantifiable insights into the neuronal electrical and chemical activities that propagate the reflex. The Hoffmann (H) reflex stands as a prototypical example, encompassing all the essential components of a reflex arc. Its presence serves as a testament to the integrity of the arc and any factors that might influence its patency [1]. Repetitive stimulation of the H-reflex enables a deeper understanding of the physiological conditions governing the reflex arc by evoking the phenomenon known as rate-dependent depression (RDD) [2]. Over the past half-century, modification of RDD has been extensively explored in conditions ranging from movement disorders to sensory sensitization. In this review, we describe the historical evolution of the H-reflex and RDD, as well as the diverse applications of RDD across various fields. Notably, we focus on its recent emergence as a tool for evaluating spinal modulation of sensory perception in animals and humans with painful diabetic neuropathy.

THE HOFFMANN REFLEX

Overview and anatomy

The H-reflex, first described by Piper [3] in 1912 and further detailed by Hoffmann in 1918 [4], is widely considered a monosynaptic reflex [5], though recent studies suggest it may incorporate additional synaptic components [6]. When a mixed peripheral nerve is stimulated, a distal-oriented impulse

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travels peripherally along motor axons to excite the target muscle, generating the M-wave. At the same time, a proximal-oriented impulse, originating from sensory afferent (Ia) fibers that project axons into the posterior horn of the spinal cord ultimately synapses with α-motor neuron cell bodies in the anterior horn of the spinal cord and returns to the target muscle via the motor axons, eliciting the H-wave (the H-reflex) (Fig. 1). Because of the greater distance travelled, the H-wave is temporally delayed relative to the M-wave. In awake humans, the M-wave exhibits a higher activation threshold than the H-wave. Thus, during incremental increasing of stimulation intensity, α-motor neurons governing slow-twitch fibers are initially activated following input from sensory afferents, contributing to appearance of the initial H-component. Subsequently, α-motor neurons controlling fast-twitch fibers become activated, leading to the emergence of the M-component [7]. As the stimulation intensity continues to climb, there is a decrease in H-amplitude.

The pattern of the H-response differs significantly from the F-response, which exhibits variable waveforms and unstable latency due to activation of different anterior horn cells. Distinguishing between the F and H responses in clinical electromyography can be challenging at times, although it should also be noted that F responses are rare in rodents. Jabre [8] established that for a response to be considered an H-reflex, it must fulfill three criteria: (1) it must occur either without an M-response or be preceded by a minimal M-response; (2) its latency must decrease when the nerve is stimulated proximally; and (3) its amplitude must decrease as the stimulation frequency increases.

The H-reflex is commonly measured in the leg, with the surface electrodes recorded at the soleus muscle at the calf [8]. It can also be recorded on the upper arm, specifically in the flexor carpi radialis muscle, although reproducibility among individuals is not as consistent as that of soleus recording [9]. Indeed, the presence of non-soleus H-reflexes, such as in the facial or distal hand muscles, is considered indicative of upper motor neuron dysfunction in various pathological conditions [10].

It is widely acknowledged that the soleus H-reflex shares the same neural pathway as the Achilles reflex with both reflecting similar clinical significance [11]. It is estimated that only 65%

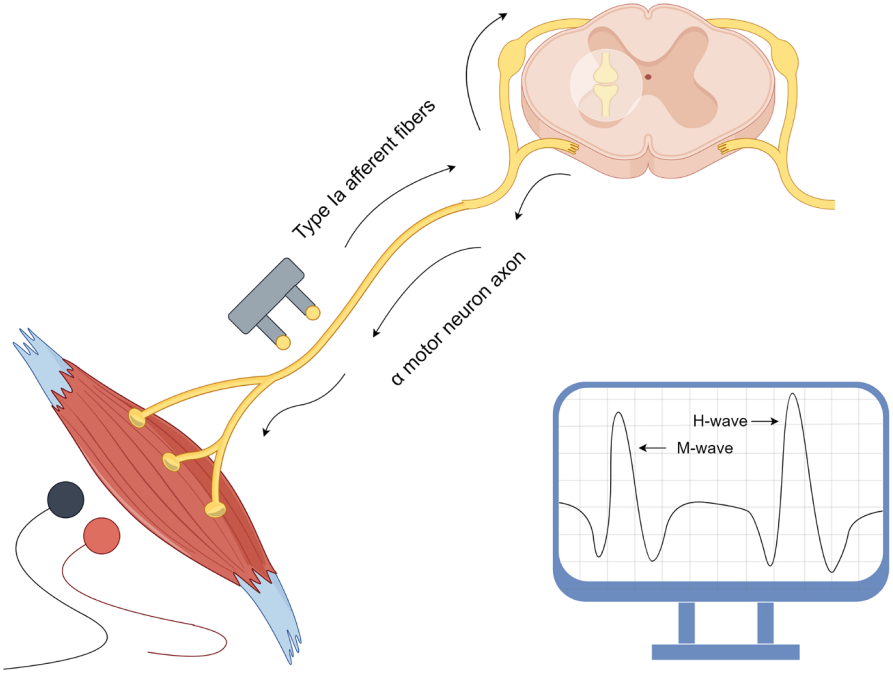

Fig. 1. The pathway and electrophysiological waveform of the H-reflex. Stimulation of large motor neurons directly excites the soleus muscle, resulting in the recording of the M-component. Excitation of the sciatic nerve is also relayed by type Ia afferent fibers through the dorsal root ganglion. Subsequently, it enters the spinal cord via the posterior root and posterior horn, establishes synapses with α motor neurons, and is propagated through their axons to evoke excitation in the soleus muscle, thereby generating the H-component.

of individuals over 60 years old retain the H-reflex, paralleling the survival rate of the Achilles reflex [12]. Despite this age-related occurrence rate, the latency of the H-reflex appears to remain unaffected [13].

Clinical applications

The H-reflex holds significant value in both neurology and rehabilitation. In humans, the sciatic nerve, which is the most common nerve stimulated to elicit the H-reflex, is innervated by the S1 segment and its corresponding root. The integrity of this neural pathway greatly impacts the occurrence and amplitude of the H-wave, underlining its significance in diagnosing S1 radiculopathy [14]. At the earliest stages of this condition, asymmetry in H-reflex amplitude between the afflicted and contralateral sides is observed rather than a prolongation of H latency [15]. Given that H-waves tend to diminish in pathological conditions, various techniques have been explored to enhance their measurement, including proximal stimulation, which has shown particularly promising results [16]. By employing a high-voltage electrical stimulator at the S1 foramen, an H-wave can be elicited, effectively shortening the afferent pathway and minimizing the impact of sensory input deficits [17]. This approach has potential applications in a wide range of diseases involving the S1 root, such as ganglionopathies [17] and diabetes mellitus [18].

An abnormal H-reflex also often serves as the earliest identifiable sign in the onset of polyneuropathies such as Guillain-Barré syndrome (GBS) [19]. Indeed, the bilateral absence of the H-reflex is the most sensitive marker for early diagnosis of GBS and its variants [20] with over 60% of patients with GBS lacking H-reflexes [21]. Conversely, increased H-reflex amplitudes and the emergence of H-reflexes in the soleus muscle have been observed in some patients with axonal GBS and hyperreflexia, suggesting heightened motor neuron excitability [22]. Impairment of the H-reflex may not therefore solely stem from peripheral nerve lesions, emphasizing the crucial role of the spinal cord.

In patients with diabetes, absence of the H-reflex was observed in approximately 40% of cases, particularly amongst older subjects who also exhibited more marked alterations in other electrophysiological parameters [23]. While complete loss of the H-wave in rodent models of diabetes has not been described, one study reported a decrease in H-wave amplitude in rats with streptozotocin (STZ)-induced diabetes that was attributed to a deficiency in Ia afferent fibers [24]. Intriguingly, it has been hypothesized that the H-wave could potentially be restored in labile or ketoacidotic diabetes [25].

Multiple modified H-reflex based techniques have been devised to analyze pathological alterations within the reflex arc (Table 1). The H_{max}/M_{max} ratio serves as a metric to approximate the proportion of motor neurons in the pool that can be activated [26], and also aids in the early detection of diabetic neuropathy [27]. Subsequently, the average H-reflex amplitude (H_{mean}) [28] and the single motor unit method [29] were devel-

Table 1. Comparison of various modified H techniques

Modified H techniques	Measurement	Utility	Application
H_{max}/M_{max}	The ratio of the maximum H-wave amplitude to the maximum M-wave amplitude in a train of stimulation	The excitability of the motor neuron pool	Movement disorders/Neuropathies (one of the earliest electrophysiological changes in diabetic peripheral neuropathy)
H_{mean}	The mean amplitude of the H-wave within the designated range of the M-wave in established coordinates by the M-wave and the H-wave	The excitability of the motor neuron pool	Movement disorders
Single motor unit H-reflex	Applying minimal stimulation to induce the H-wave elicited by discharge of single motor units, akin to motor unit number estimation	The excitability of the motor neuron pool	Movement disorders
Vibratory inhibition of the H-reflex	Changes in the H-reflex during vibration	(Presynaptic) Spinal inhibition	Movement disorders
Conditioned H-reflex	Using paired stimulus paradigm of low and high intensity	(Postsynaptic) Spinal inhibition	Movement disorders
Rate-dependent depression	Repetitive stimulation of the H-reflex when maximum H-wave is reached	(Pre- and/or postsynaptic) Spinal inhibition	Movement disorders/Neuropathies (identification of spinal pain generator site in diabetes for personalized therapy)

oped to serve similar roles. Techniques such as H-reflex vibration inhibition [30], conditioned methods, and RDD (discussed in detail below) are considered useful for evaluating both presynaptic and postsynaptic inhibitory functions. Indeed, these refined H-reflex techniques were initially applied primarily in movement disorders such as spasticity and amyotrophic lateral sclerosis (ALS), rather than in peripheral neuropathies. However, they have demonstrated the value of the H-reflex in assessing synaptic inhibition, providing a theoretical basis for expanding its application range.

RATE-DEPENDENT DEPRESSION OF THE H-REFLEX

Origins of the RDD technique

In 1966, Ishikawa et al. [2] introduced a technique that measured the amplitude variation of the H-reflex under continuous stimulation that has been variously termed RDD, frequency dependent depression/inhibition, rate-sensitive/dependent inhibition, and other related terms (Fig. 2). This initial study encompassed both healthy individuals and those with spinal cord injuries (SCI). Subjects were stimulated at 1 and 10 Hz to elicit the H-reflex. H-wave attenuation during repeated stimulation in healthy individuals mirrored that previously reported in animals. However, the decline in H-wave amplitude varied with the intensity of spasticity [2], suggesting that the potential is influenced by a component within the monosynaptic reflex pathway. Initially, this phenomenon was attributed to homosynaptic depression due to transmitter depletion, or inconsistencies in motor input [31]. Subsequent investigations revealed that synaptic depression could persist for 10 to 400 ms post-stimulation [32] and was unaffected by age within certain stimulation intervals [33]. Therefore, repeatedly eliciting the H-reflex at an appropriate frequency can effectively reflect the characteristics of synaptic inhibition [34]. RDD has subsequently been extensively explored in humans and rodents with movement disorders, including those with SCI, post-stroke spasticity, and also in locomotor exercise assessment.

Spinal inhibition and RDD

One interpretation of the RDD phenomenon is that the decrease in the amplitude of the H-reflex observed following

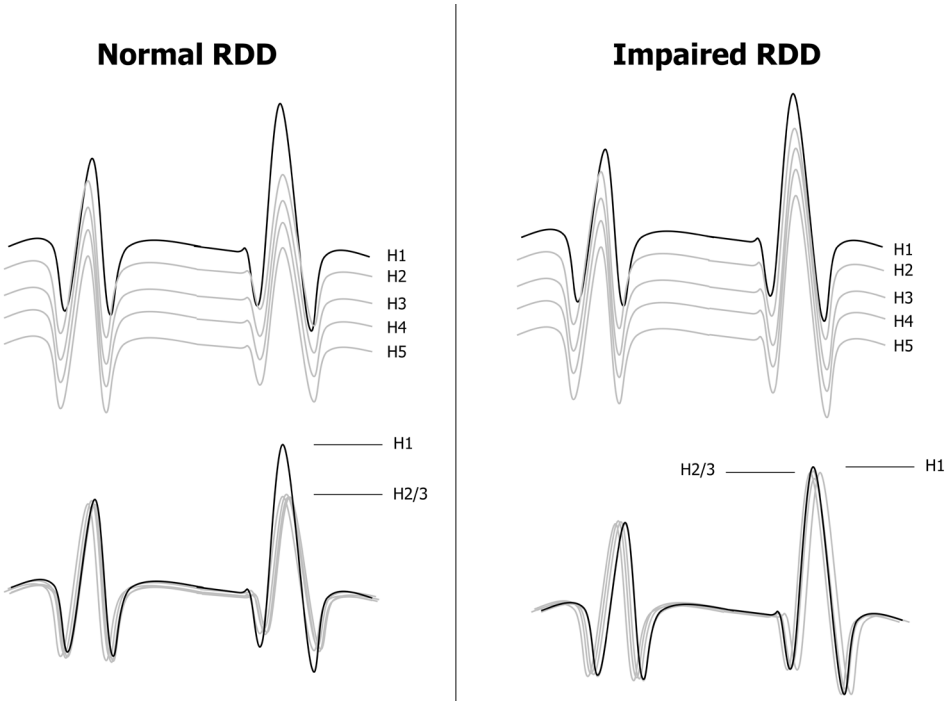

Fig. 2. Schematic diagram of waveforms during normal and impaired rate-dependent depression (RDD). Following administration of a series of five consecutive H-stimulations (H1 to H5), a distinct attenuation of the amplitude of H2 and subsequent waves compared to H1 becomes evident. This phenomenon is referred to as RDD. In certain pathological conditions, this attenuation disappears.

consecutive stimulations reflects a protective response against potential harm. This response is intricately linked to inhibitory mechanisms within the central nervous system (CNS), whose anatomical organization at the spinal level requires consideration. Peripheral sensory afferents do not establish a direct link with the brain but synapse with ascending second order spinal neurons or, in the case of reflex arcs, with motor neuron cell bodies. These synapses are prone to fatigue [35] and exhibit significant plasticity [36]. Regulation of spinal synaptic transmission is achieved through various synaptic processes such as inhibition, facilitation, modulation, and plasticity. The spinal inhibition system comprises both presynaptic and postsynaptic inhibition with a particular focus on inhibition mediated by γ-aminobutyric acid (GABA) interneurons [37]. Two extrasynaptic GABA receptors, $GABA_AR$ and $GABA_BR$, play crucial roles. $GABA_AR$ functions as a typical chlorine (Cl^-) channel [38], whereas the inhibitory function of the $GABA_BR$ is mediated through G-protein-coupled potassium (K^+) efflux and calcium (Ca^{2+}) influx [39]. Recently, the contribution of Cl^- balance to membrane excitability has received particular attention [40] as Cl^- influx leads to hyperpolarization while its outflow can result in depolarization. The Cl^- distribution at resting state is maintained by the membrane pumps potassium-chloride-cotransporter 2 (KCC2) which pumps Cl^- inward, and sodium-potassium-chloride-cotransporter 1 (NKCC1) which performs the opposite function. KCC2 and NKCC1 thus contribute to a dynamic Cl^- balance, determined by relative expression and activity of the two pumps. Notably, primary afferents express predominantly NKCC1 so exhibit a relatively high internal [Cl^-] at rest, whereas KCC2 dominates in spinal postsynaptic neurons which exhibit a relatively low internal [Cl^-] at rest [41]. This balance of Cl^- pump expression/activity sets the local Cl^- equilibrium potential and is essential for the normal functioning of $GABA_AR$ [42]. Consequently, upon activation by GABA, postsynaptic $GABA_AR$ facilitates Cl^- influx along its concentration gradient, leading to hyperpolarization and the initial phase of the inhibitory postsynaptic potential [43] that in part confers the generally accepted inhibitory status on spinal GABA and its agonists.

RDD has been postulated to originate from "homosynaptic depression," which occurs through an intrinsic, homosynaptic mechanism within a single synapse that manifests as a gradual reduction in the amplitude of postsynaptic potentials under consecutive presynaptic stimulations [44]. While the exact mechanism of homosynaptic depression remains elusive, it is hypothesized by some researchers to be presynaptically-driven. However, this assertion remains controversial as GABA receptors also play a postsynaptic role and other factors might impact RDD. The actual scenario is likely to be intricate and complex.

RDD IN MOVEMENT DISORDERS

As noted above, the RDD technique emerged largely through meticulous studies on SCI. While there are occasional reports suggesting normal RDD [45], the majority of published research overwhelmingly supports the finding that RDD is compromised in SCI, both in animal models and humans [46,47]. Loss of RDD was reported in rats displaying spasticity and rigidity following ischemic SCI, with spasticity overcome by spinal delivery of GABA agonists [48]. An intriguing clinical study postulated that loss of RDD in SCI originates from impaired presynaptic mechanisms as motor-evoked potential responses, purportedly reflecting postsynaptic inhibition, were comparable among subjects with and without RDD [49]. However, the pure presynaptic origin theory of spasticity is not unanimously embraced, as other studies implicate glycinergic inhibition of lumbar motor neurons in the mechanisms underlying spasticity after SCI, which also influences RDD [34,50]. Further mechanistic associations were provided by Boulenguez et al. [34] who reported that blocking KCC2 in healthy rats diminished RDD while RDD was also reduced in both KCC2-deficient mice and intact rats following intrathecal delivery of brain-derived neurotrophic factor (BDNF) injection to downregulate KCC2. This data buttresses the association between RDD and the KCC2-related inhibitory system, which may involve both GABAergic and glycinergic neurons. It also stands out as one of the few studies to demonstrate RDD using isolated tissues [34,51].

Advancements in medical technology and the emergence of targeted therapies has caused RDD to develop into a mature tool for assessing drug efficacy in SCI and spasticity. These treatments encompass diverse methods including exercise training [52-54], spinal electric or electromagnetic stimulation [55-57], nerve signal conduction blockade, adipose tissue-derived stromal vascular fraction [58], α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonists [59], adeno-associated virus encoding human neurotrophic factor 3 [60], delayed injection of physically cross-linked PNIPAAm-g-PEG hydrogel [61], L-Dopa [62],

and the KCC2 activity enhancer CLP257 [63]. RDD was also applied in the exploration of astrocytes and motor neuron dendritic spines as crucial mediators in SCI [64-66] and to assess the utility and consistency of other evaluation metrics such as swimming tests [67]. RDD is not simply an indicator of certain neurological insults arising from SCI but may also be applied to other movement disorders, especially those accompanied by spasticity. It has been assessed in models of cerebral palsy [68], post-stroke spasticity [69-71], hyperkinesis [72], multiple sclerosis, and Parkinson's disease [73,74]. In patients with ALS, RDD impairments have been observed and correlated with motor unit size [75], though the clinical link between RDD and upper motor neuron impairment in this disorder remains unexplored.

RDD IN PAINFUL DIABETIC PERIPHERAL NEUROPATHY

A shift in perspective towards sensory evaluations

In 2021, the International Diabetes Federation reported that there were an estimated 537 million persons suffering from diabetes worldwide and predicted that this number will grow to 784 million by 2045 [76]. The vast majority have type 2 diabetes mellitus (T2DM) in which hyperglycemia is secondary to insulin resistance, with a smaller fraction having insulin-deficient type 1 diabetes mellitus (T1DM). The life expectancy of people with diabetes is significantly reduced [77], in large part due to progressive damage to multiple organ systems in what are termed the "complications" of diabetes. Neuropathy is the most common of the diabetic complications, occurring in 20% to 70% of persons with diabetes surveyed depending on population characteristics and method of diagnosis [78,79]. Diabetes and its metabolic consequences may impact both the peripheral nervous system (PNS) and as is more recently appreciated, the CNS. Neuropathy is also detected in persons with pre-diabetes and metabolic syndrome, representing further large populations worldwide [80]. The most common presentation of diabetic neuropathy is as a distal symmetrical polyneuropathy with sensory loss as the dominant feature. Pain is a frequent feature, with over 30% of T2DM surveyed at home describing some painful symptoms, including a sub-set who were yet to be diagnosed with neuropathy [81]. There is currently no treatment for diabetic neuropathy that is approved by major regulatory agencies such as the European Medicines Agency and the United States of America Food and Drug Administration, with guidance limited to improving glycemic control and use of analgesics or medical devices to suppress pain. Unfortunately, maintaining consistent normoglycemia is not viable for most diabetics and is increasingly considered ineffective in patients with T2DM. Analgesics frequently cause undesirable side effects and do not treat the underlying neuropathy. There remains an urgent need for effective pharmacotherapies to manage both diabetic neuropathy its associated pain state.

Neuropathic pain has become a focal point in the management of peripheral neuropathy in recent decades with diagnosis and treatment guidelines including the European Academy of Neurology joint guidelines [82], Neuropathic Pain Special Interest Group recommendations [83], and National Institute for Health and Care Excellence guidelines [84]. These guidelines consistently recommend gabapentinoids (gabapentin, pregabalin and related molecules) along with serotonin and norepinephrine reuptake inhibitors, tricyclic antidepressants, and other analgesic drugs. However, recent opinions suggest that the effectiveness of neuroanalgesics is overestimated [85, 86]. Moreover, the number needed to treat to achieve a 50% analgesic effect with first-line drugs like gabapentin and pregabalin in clinical trials is typically 4 to 5 or higher [86-88], indicating that the drugs may be effective in only 20% of patients. Current analgesics also frequently produce harmful and disruptive side effects such as addiction and sedation and do not address the underlying pathogenesis of the pain.

Multiple biometric methods have been employed in both clinical [89] and experimental [90] settings for the diagnosis and staging of diabetic neuropathy and for assessment of efficacy for potential therapeutics. Assays can be broadly categorized into those evaluating large nerve fibers, encompassing nerve conduction studies and nerve biopsies, and those targeting small sensory fibers, such as quantitative sensory testing and assessment of nerve pathology in skin biopsies or corneal nerve images. These measurements, along with assays of autonomic dysfunction are frequently combined into composite scoring systems such as the Toronto Clinical Neuropathy Score and the Michigan Diabetic Neuropathy Score, which are valuable for identifying neuropathy in patients with overt diabetes as well as impaired glucose tolerance [91]. Scales that emphasize patient reported outcomes such as the Norfolk Quality of Life questionnaire may also be modified to target diabetic neuropathy [92]. While sensory loss is frequently the primary focus of these scales, pain is usually incorporated using a combi-

nation of qualitative descriptors and numerical ratings such as the visual analog scale (VAS). However, current clinical measurement systems have not yet advanced to incorporate the emerging appreciation that pain need not have its pathogenic origins at the site of pain perception [79]. Absent such discrimination, development of new targeted pain therapeutics cannot proceed with the required precision.

It is becoming increasingly recognized that the pain component of diabetic neuropathy may originate from one or more of multiple potential mechanisms that operate concurrently, with one mechanism dominant at any given time but in which the order of precedence may shift as the underlying degenerative neuropathy progresses [93,94]. There have been recent efforts to provide a detailed phenotyping of pain in diabetic subjects that may have implications for the underlying pathogenic mechanisms involved [95] and shifts in phenotype over time have recently been described [96]. The diverse nature and location of these pain mechanisms suggests a need for personalized treatment approaches and drug selection [97]. Consequently, the same analgesic may exhibit widely varying efficacy among patients with different pain generating mechanisms. This mechanistic heterogeneity was recognized in a clinical trial of an agent that targeted peripheral T-type calcium channels, in which the study cohort was enriched with subjects in whom pain was associated with abnormal spontaneous activity of C-fibers as measured by microneurography [98]. Unfortunately, the absence of reliable, less invasive and time-consuming biological markers to definitively identify the primary pain mechanism in individual patients hinders our ability to effectively guide clinical decisions in selecting the most appropriate analgesic drugs.

Many of the physiological and pathophysiological mechanisms of sensory disorders are potentially analogous to those of motor disorders, particularly in their regulation at the spinal cord level by KCC2-mediated Cl^- balance and GABAergic or glycinergic-mediated inhibition [99,100]. Indeed, chronic neuropathic pain has been associated with both direct damage to C-fibers and KCC2-associated pathological amplification of

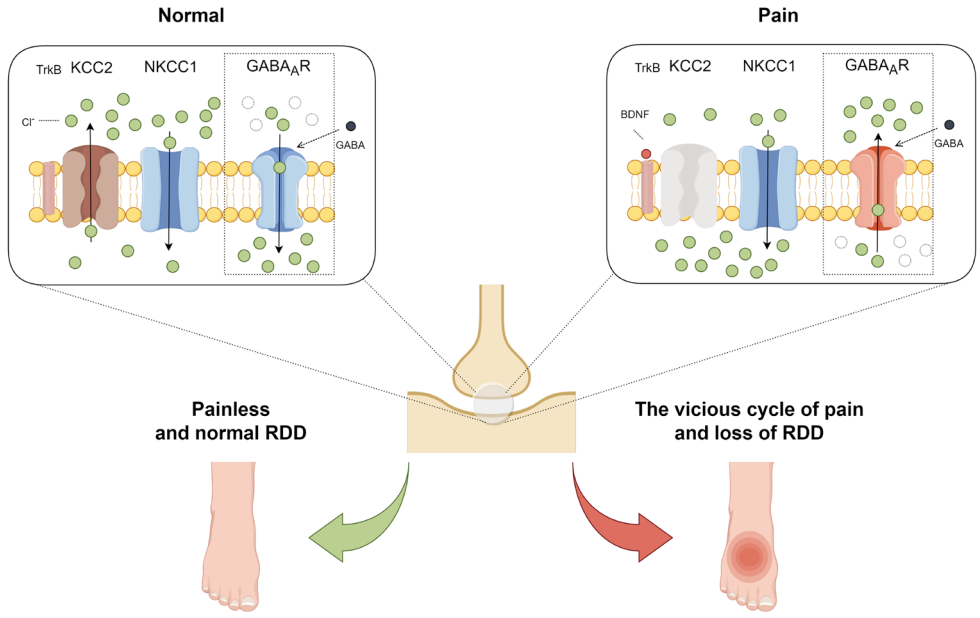

Fig. 3. Critical membrane proteins and ion flows of GABAergic synaptic modulation. Potassium-chloride-cotransporter 2 (KCC2), responsible for mediating chlorine $(Cl)^-$ efflux, and sodium-potassium-chloride-cotransporter 1 (NKCC1), mediating Cl^- influx, jointly maintain the Cl^- balance of the cell membrane. γ-Aminobutyric acid receptor A ($GABA_AR$), serving as a Cl^- channel, when interacts with GABA in a healthy state, leading to Cl^- influx and subsequent cellular hyperpolarization. The inhibitory regulation of this ensemble of membrane proteins is hypothesized to be a key mechanism underlying rate-dependent depression (RDD). In pathological conditions, the downregulation of KCC2 expression induced by brain-derived neurotrophic factor (BDNF) alters the intracellular and extracellular $[Cl^-]$, while the function of $GABA_AR$ relies on $[Cl^-]$, leading to the efflux of Cl^- and tending to depolarization, thus intensifying pain. The impairment in this regulatory system alters the postsynaptic currents, ultimately manifesting as the absence of RDD. TrkB, tyrosine kinase receptor B.

signal at the spinal cord level [101] by mechanisms involving BDNF and its receptor tyrosine kinase receptor B (TrkB), KCC2, and GABAergic neurons [102]. As discussed above, in healthy individuals with functional neuronal KCC2 pump activity and thus relatively low internal [Cl^-], $GABA_AR$ functions as an inhibitory Cl^- channel as GABA binding opens the channel and Cl^- flows into the neuron. However, under pathological conditions that impair KCC2 pump activity and thus elevate internal [Cl^-], the flow of Cl^- is reversed upon GABA binding to $GABA_AR$. Consequently, GABA acting through $GABA_AR$ is no longer inhibitory and may indeed become excitatory. In diabetic rodents, it has been suggested that reversal of $GABA_AR$ function, coupled with increased spinal GABA [103] contributes to a vicious cycle in neuropathic pain (Fig. 3) [104]. The relationship between these factors and the mechanisms of RDD is detailed below.

Preclinical evidence for RDD defects as a biomarker for spinal sensitization

As the H-reflex pathway incorporates motor, spinal and sensory components a compromised RDD could indicate dysfunction in any combination of these systems, via their synaptic regulatory mechanisms, interneurons and descending pathways [75]. However, it is also generally acknowledged that diabetes does not impede the central regulation of movement [105]. Thus, when considering the impact of diabetes on RDD, focus shifts to potential disruption of sensory input and spinal synaptic regulation.

The initial discovery of impaired RDD in diabetes was made using the STZ-induced diabetic rat model of T1DM [106]. This finding has been confirmed in subsequent studies [104,107-110] and in STZ-induced diabetic mice [111]. The disorder is not a toxic effect of STZ [107] and has also been observed in rodent models of T2DM and of metabolic syndrome [107,112]. These rodent models of diabetes do not develop spasticity or other movement disorders, so that the subsequent investigational focus was on sensory and spinal components of the RDD pathway. Studies of spinal pharmacology in normal rats showed that the application of the $GABA_AR$ antagonist bicuculline eliminated RDD [106], indicating that the GABAergic inhibitory system contributes to normal RDD function. In contrast, application of bicuculline to the spinal cord of diabetic rats did not ablate RDD but restored diminished RDD back to control values [106,110]. This suggests that under diabetic conditions $GABA_AR$ activity impairs RDD rather than drives it. Notably, spinal bicuculline also alleviated tactile allodynia in the same diabetic animals, implicating $GABA_AR$ activity in genesis or maintenance of diabetes-induced neuropathic pain. These observations provided the first evidence of a convergence point for pathways that (1) drive RDD and (2) modulate spinal pain processing and prompted the speculation that RDD deficits could be used as an electrophysiology biomarker to identify neuropathic pain states in which a component of the pathogenic mechanism derived from spinal GABAergic dysfunction.

As noted above, the inversion of $GABA_AR$ function in pathological conditions is underpinned by an imbalance of neuronal Cl^- homeostasis due to impaired anion extrusion. The anion transporter KCC2 is widely expressed in the mature CNS, including spinal neurons, whereas expression is minimal [113] or absent [114] mature primary afferent neurons of the PNS. Thus, neuronal Cl^- homeostasis on the presynaptic side of the spinal synapse is largely regulated by the highly expressed NKCC1 pump, while KCC2 dominates the postsynaptic site [115]. In the spinal cord of diabetic rodents expression of NKCC1 protein remains unchanged, whereas expression of KCC2 protein decreases [106]. Moreover, the KCC2 inhibitor dihydroindenyl oxy alkanoic acid (DIOA), ablates RDD in normal rats and mimics other aspects of diabetes, namely subsequent restoration of RDD by bicuculline and the induction of behavioral indices of neuropathic pain. This suggests that KCC2 deficits participate in disruption of RDD upstream of $GABA_AR$ [104]. The immediate cause of impaired spinal KCC2 expression (and presumably activity) in diabetes is linked to spinal BDNF. Throughout the nervous system KCC2 expression is negatively regulated by BDNF, which acts via the TrkB receptor to control both channel transcription and lysosomal degradation [116]. Spinal delivery of BDNF to normal rats reduces KCC2 expression and, like pharmacological KCC2 inhibition with DIOA, ablates RDD and triggers behavioral indices of neuropathic pain [104]. Thus, three conditions where spinal KCC2 is reduced; excess BDNF, direct KCC2 inhibition and diabetes share common consequences in both impaired RDD and induction of neuropathic pain. Further support for the pathogenic role of spinal BDNF in loss of RDD comes from studies in which sequestration of spinal BDNF in both male and female diabetic rats restored RDD and alleviated neuropathic pain [104]. These studies suggest that impaired KCC2 activity contributes to the mechanisms of both RDD loss and neuropathic pain in diabetic rodents. They also implicate ele-

vated spinal BDNF as an initiating pathogenic mechanism, which is consistent with its widespread but complex involvement in a variety of neuropathic pain models [117]. Excess BDNF may derive from activated spinal microglia, which have been reported in diabetic rodents and suggested as a source of the increased spinal BDNF mRNA expression described in these animals [118,119]. Primary afferents also express BDNF and export it to central terminals by anterograde axonal transport [120]. Increased BDNF mRNA and protein expression in dorsal root ganglia of diabetic rats [108,118,121] could therefore also result in increased release into the spinal cord during primary afferent activity (Fig. 4).

Other than the source(s) of spinal BDNF, several other issues remain to be resolved regarding the mechanisms of RDD attenuation during diabetes. While the restriction of loss of spinal KCC2 protein to the dorsal horn of diabetic rodents [104,110] is consistent with accepted models of anatomic modulation of ascending sensory processing it also implies a first synapse of the RDD reflect arc within the dorsal horn. This is controversial, as the RDD pathway has been widely considered a monosynaptic reflex with the synapse activating motoneuron cell bodies in the ventral horn. Arguments have been advanced that the H-wave has properties consistent with contributions from an oligosynaptic pathway [7,108] although such a pathway has yet to be anatomically mapped. Consideration of the synapse also raises the issue of whether loss of RDD arises from presynaptic or postsynaptic disorders, given that $GABA_A$ receptors are distributed across both presynaptic and postsynaptic neuronal membranes [122]. The post-synapse is an appealing candidate, due to the marked expression of KCC2 in spinal neurons combined with reports of either low [113] or absent [114] KCC2 mRNA and/or protein expression in primary afferents. However, an influence of presynaptic KCC2 or other Cl^- extruding DIOA-sensitive channels such as KCC3 [123] concentrated to a pertinent sub-set of primary afferents cannot be discounted. Further, the involvement of extra-synaptic $GABA_A$ receptors containing α5-$GABA_A$ sub-units has been suggested in a variety of neuropathic pain states, including diabetes [109,124]. This sub-type is localized to extra-synaptic regions of both pre- and postsynaptic neurons in the spinal cord

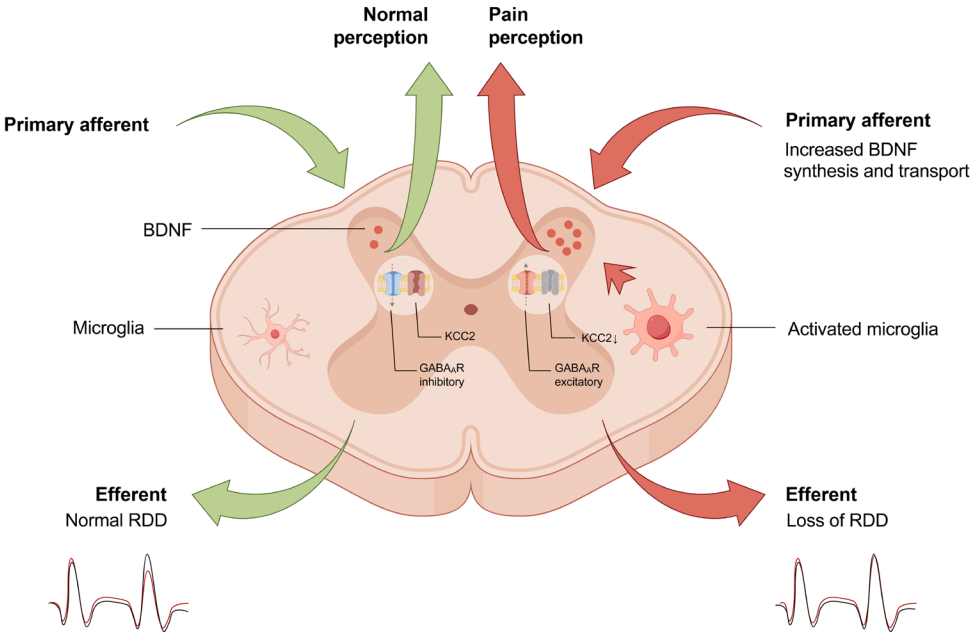

Fig. 4. The correlation between the inhibitory regulatory dysfunction of the ascending sensory pathway at the spinal cord level and the rate-dependent depression (RDD) output. This exploration illustrates the scientific hypothesis of the normal ascending sensory pathway through the spinal cord (green arrows) and the ascending sensory pathway during pain conditions (red arrows). It is postulated that the original afferent inputs and activated glial cells may jointly contribute to the abnormal increase in brain-derived neurotrophic factor (BDNF), which subsequently leads to the downregulation of potassium-chloride-cotransporter 2 (KCC2) and the conversion of γ-aminobutyric acid receptor A ($GABA_AR$) into excitatory. This process results in the disinhibition of the spinal cord, manifesting phenotypically as pain and hyperalgesia, and electrophysiologically as impairments in RDD.

and, in normal animals, modulates a tonic inhibitory system [125]. As diabetes also increased expression of the α5-$GABA_A$ subunit, it was proposed that loss of RDD and allodynia are secondary to hyperactivity of α5-$GABA_A$ receptors acting in their excitatory mode. This could be augmented by the increase in spinal basal and stimulus-evoked GABA release seen in diabetic rats [103], which may reflect a residual attempt to suppress pain by GABAergic inhibitory systems, or GABA release from activated astrocytes [126]. Finally, RDD status can also be modulated by spinal $GABA_B$ receptors whose ongoing inhibitory function can transiently mask loss of $GABA_A$ mediated inhibition [110].

The proposed convergence of pathogenic mechanisms leading to RDD deficits and neuropathic pain arising from spinal disinhibition in diabetes has prompted the suggestion that correcting the former may predict efficacy of agents against the latter. As noted above, acute pharmacological interventions with $GABA_A$ antagonists or BDNF sequestration both restores normal RDD in diabetic rats and alleviates behavioral indices of neuropathic pain. Direct enhancers of KCC2 exist and alleviate neuropathic pain in some models [127,128] although whether they impact both RDD and pain in models of painful diabetic neuropathy has yet to be reported. Alleviation of impaired RDD and pain also coincides in diabetic rats following intrathecal delivery of acetazolamide, a carbonic anhydrase inhibitor that blocks bicarbonate efflux to counteract disruption of the chloride gradient arising from reduced KCC2 [110]. Conversely, agents that are used clinically to alleviate pain via actions at the spinal level such as the 5-hydroxytryptamine 2A inhibitor duloxetine and gabapentin also restore RDD [107, 129,130]. Other than acute pharmaceutical interventions, extended treatment with insulin at doses that do not reduce hyperglycemia or C-peptide also restore RDD and alleviate indices of painful neuropathy in rodent models of diabetic neuropathy, potentially via their neurotrophic effects [107,111]. It will be important to determine whether these observations translate into an ability to use efficacy in restoring normal RDD in clinical development programs for drugs targeting painful diabetic neuropathy.

RDD in diabetic patients

It is becoming increasingly appreciated that mechanisms of neuropathic pain can be intricate and dynamic, involving regulation of sensory functions at the peripheral, spinal, and cerebral levels that evolve in their relative importance and interactions over time. A further challenge is that pain may have diverse origins within a given primary pathogenic condition, despite similar phenotype and presentation. This complexity may underlie practical clinical experience where there are marked variations in efficacy of analgesic drugs against pain with similar qualities, presentations and initiating pathogeneses. One approach to addressing this complexity is to identify biomarkers that give insight into the generator site of pain in a specific patient at a specific time in their disease progression, to guide choice of most pertinent medication. The preclinical studies described above suggest that RDD may serve as such a biomarker, prompting investigation of potential translation to the clinical setting.

Although there are currently no large-scale studies in healthy populations, RDD has been measured in the control groups of multiple disease-orientated studies, with various frequencies, measurement, and calculation methods explored. The current consensus considers 1 Hz as the most appropriate stimulus frequency within the 0.2 to 10 Hz spectrum as between-group differences in RDD are less dramatic at lower frequencies [107,131]. RDD is typically defined as the ratio of the nth H-wave (Hn) amplitude to the first H-wave amplitude, with the choice of nth wave being somewhat arbitrary. Studies in diabetic subjects reported that the clearest discrimination from control subjects was achieved when RDD was calculated using H3 to H1 ratio [107,130,132] while using the average amplitude of the stimulus sequence can also effectively distinguish patients with or without spinal disinhibition [131-133].

RDD in healthy populations manifests as an approximately 30% attenuation of H-wave amplitude [107,130,134]. RDD is impaired in both T1DM [107] and T2DM subjects [134] and also in subjects with pre-diabetes [131]. Subgroup analyses suggest that regardless of T1DM or T2DM, individuals with painful diabetic neuropathy are more likely to exhibit impaired RDD compared to those with equivalent neuropathy but without pain [107,130-134]. Interestingly, this impairment is not correlated with large or small fiber (skin or corneal) assessments in diabetes [107,130,134], suggesting that tissue damage may not be the sole mechanism of pain. A recent deep phenotyping of pain in subjects with diabetes and pain found that patients who exhibit higher pain scores or specific sensory threshold abnormalities such as mechanical pain sensitivity and heat hyperalgesia tend to have more severe RDD impairment [107,133]. This suggests a unique pain phenotype representing spinal cord inhibitory disorder that may be distin-

guished from other pain phenotypes in diabetic subjects by marked RDD loss. Most intriguingly, enhancement of RDD beyond normative values in control subjects was seen in some diabetic patients without pain [132,134], although this phenomenon is not universal [131] and has yet to be fully explored. Together, recent studies suggest that impaired RDD is not a marker for type or severity of diabetes or neuropathy *per se* but a reflection of the dominance of a specific pain mechanism, spinal inhibitory dysfunction, within a subgroup of subjects with diabetes, neuropathy and pain.

Clinical application of RDD in painful diabetic neuropathy

Measurement of RDD in the clinical setting represents a simple, quick and potentially inexpensive modification of the nerve conduction protocols commonly used in neurology clinics to investigate many forms of peripheral neuropathy, including diabetic neuropathy. As this electrophysiological procedure is non-invasive and anesthesia-free it is well tolerated by patients. Once normative values for RDD are established and available for reference, detection of impaired RDD may inform several subsequent clinical choices. For example, the subgroup of patients, in which impaired RDD suggests ongoing spinal disinhibition as a contributor to neuropathic pain, may respond better to treatments that target spinal systems, such as duloxetine, rather than agents that impact hyperactive peripheral nociceptors. Thus, RDD screening offers the beginning of a personalized medicine approach to treating painful diabetic neuropathy. At present, while preclinical studies indicate that duloxetine has therapeutic effects on pain [129] and RDD deficits [107], no clinical studies have yet investigated whether diabetic patients with impaired RDD show pain that is particularly responsive to duloxetine. In contrast, a recent study correlated pain and RDD function during treatment with gabapentin. Gabapentin was designed as a GABA mimic yet has a low affinity for both the $GABA_AR$ and $GABA_BR$ and does not directly influence the function of $GABA_AR$ [135]. It has become generally accepted that gabapentin exerts its analgesic effect through interaction with upregulated α2δ-1 subunit of voltage-gated calcium channels in the spinal cord during neuropathic pain states and is independent of GABAergic function [136]. In an interventional study lasting two weeks in patients with symptomatic painful diabetic neuropathy (VAS ≥3), it was found that as treatment progressed, the anticipated improvement in pain scores (VAS) was accompanied by reversal of impaired RDD. A 1% decrease in RDD was associated with a 1.27-fold increase in benefit against pain [130]. This suggest a predictive value of RDD that could guide drug decision-making based on an objective indicator. Whether RDD deficits respond to non-GABA related analgesics such as pregabalin, ziconotide, and third-generation gabapentinoids like crisugabalin and mirogabalin awaits further investigation, as does the mechanism by which gabapentin reversed the RDD deficit.

CONCLUSIONS AND FUTURE CHALLENGES

Tracing the history of RDD though its discovery, exploration, and application, reveals a distinct evolution from motor to sensory applications. The initial utility of RDD centered on movement disorders, whereas its more recent applications have presented it as a biomarker capable of reflecting spinal sensory processing. By identifying individual patients with impaired RDD and thus a presumed contribution of spinal disinhibition to neuropathic pain, RDD has the potential to guide analgesic prescription strategies for patients with painful diabetic neuropathy based on mechanisms of pain and drug actions (Fig. 5). It may also have value in selecting subjects for clinical trials of analgesics that target painful diabetic neuropathy, by either inclusion if the agent has a relevant spinal mechanism of action or exclusion if the target is peripheral. This approach to selecting analgesic medications based on objective indicators is anticipated to be more efficient and precise than traditional methods that rely on subjective experience.

Preclinical research indicates that the GABAergic system plays a pivotal role in RDD. Other pathways, including the glycinergic systems and the α2δ-1 subunit could also potentially impact spinal processing of RDD at either the pre- or postsynaptic levels, reflecting a comprehensive and interconnected synaptic regulation that awaits detailed description. To date, study of any role of glycinergic neurons in RDD has been limited by interference with the movement system in experimental animals and clinical translations that did not fully align with expectations [137]. The diagnostic potential of RDD has yet to be adopted into standard clinical practice. Practical consideration such as variability between different stimulus trains and operators require further examination and a standardized procedure and measurement system codified to allow production of normative values and an agreed definition of abnormal RDD. Another significant current limitation to adoption of RDD as a clinical biomarker of spinal GABAergic function is the attenuation and ultimate loss of the H-wave in the late

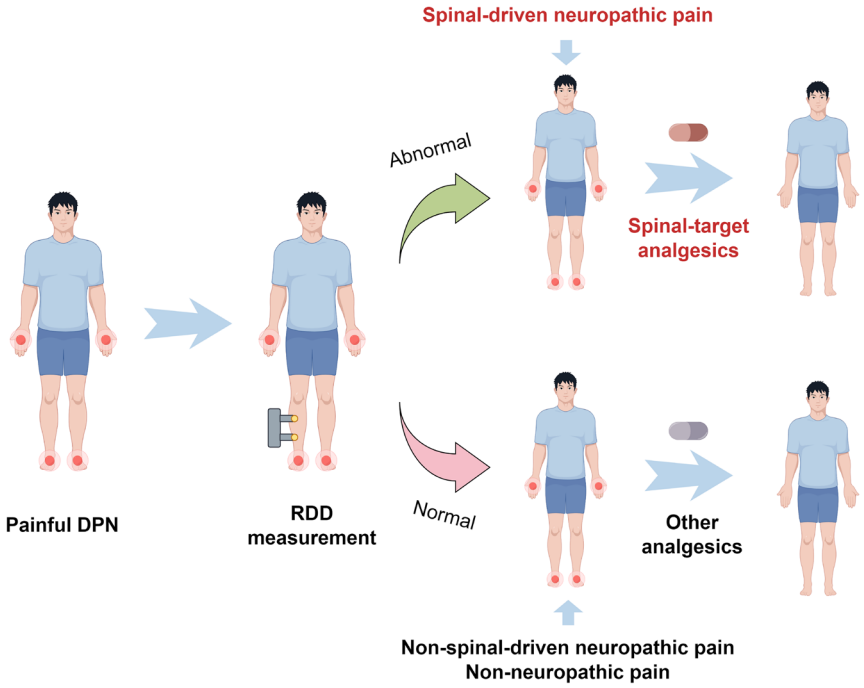

Fig. 5. Objective strategy for the analgesic prescription for patients with painful diabetic peripheral neuropathy (DPN). When individuals seek treatment, rate-dependent depression (RDD) is first assessed. Depending on whether RDD is impaired—indicating that the pain may be due to spinal target mechanisms—different medications can be selected accordingly. Unlike strategies based on anecdotal evidence, using RDD in painful DPN aims to create an objective, criterion-based targeting approach. This method detects spinal-mediated inhibitory modulation in patients with pain, allowing for the administration of tailored analgesic drugs. As a result, it enables personalized and precise analgesic therapy.

stages of human diabetic neuropathy. This limits the population amenable to use of RDD to those who retain the H-wave—generally those with mild-moderate neuropathy. Whether this can be circumnavigated by modifications to RDD technique, such as use of S1 root stimulation to enhance the occurrence of the H-wave in subjects with more advanced degenerative peripheral neuropathy, remains to be determined.

CONFLICTS OF INTEREST

Nigel A. Calcutt has been international editorial board member of the *Diabetes & Metabolism Journal* since 2024. He was not involved in the review process of this article. Otherwise, there was no conflict of interest.

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