Voluntary activation of muscle in humans: does serotonergic neuromodulation matter?

Justin J Kavanagh and Janet L Taylor
DOI: 10.1113/JP282565

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The following individual(s) involved in review of this submission have agreed to reveal their identity: Simon A Sharples (Referee #1); Gregory EP Pearcey (Referee #2); Tuan V Bui (Referee #3)

Review Timeline:

| Event              | Date       |
|--------------------|------------|
| Submission Date    | 31-Mar-2022|
| Editorial Decision | 07-Jun-2022|
| Revision Received  | 21-Jun-2022|
| Accepted           | 12-Jul-2022|

Senior Editor: Laura Bennet
Reviewing Editor: Jean-Claude Béïque

Transaction Report:
(Notes: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)
Dear Dr Kavanagh, Re: JP-TR-2022-282565 "Voluntary activation of muscle in humans: does serotonergic neuromodulation matter?" by Justin J Kavanagh and Janet L Taylor Thank you for submitting your Topical Review to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 3 expert referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision. The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made. NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors’ comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document. I hope you will find the comments helpful and have no difficulty in revising your manuscript within 4 weeks. Your revised manuscript should be submitted online using the links in Author Tasks Link Not Available. This link is to the Corresponding Author’s own account, if this will cause any problems when submitting the revised version please contact us. You should upload: - A Word file of the complete text (including any Tables); - An Abstract Figure, (with accompanying Legend in the article file) - Each figure as a separate, high quality, file; - A full Response to Referees; - A copy of the manuscript with the changes highlighted. - Author profile. A short biography (no more than 100 words for one author or 150 words in total for two authors) and a portrait photograph of the two leading authors on the paper. These should be uploaded, clearly labelled, with the manuscript submission. Any standard image format for the photograph is acceptable, but the resolution should be at least 300 dpi and preferably more. You may also upload: - A 'Cover Art' file for consideration as the Issue's cover image; - Appropriate Supporting Information (Video, audio or data set https://jp.ms submit.net/cgi-bin/main.plex? form_type=display_requirements#supp). To create your ‘Response to Referees’ copy all the reports, including any comments from the Senior and Reviewing Editors into a Word, or similar, file and respond to each point in colour or CAPITALS. Upload this when you submit your revision. I look forward to receiving your revised submission. Yours sincerely, Professor Laura Bennet Senior Editor The Journal of Physiology https://jp.ms submit.net http://jp.physoc.org The Physiological Society Huxley House 30 Farringdon Lane London, EC1R 3AW UK http://www.physoc.org http://journals.physoc.org Referee #1: Kavanaugh and Taylor provide a timely review with the goal of highlighting and summarizing recent work in humans that describe roles and underlying mechanisms for 5HT and target receptors in the voluntary activation of muscle in humans. To accomplish this end, they place their human work in the context of multiple animal models that describe how serotonin modulates intrinsic properties and ion channels of motoneurons. The review draws the conclusions that studying 5HT control of muscle activation in humans might not be as straightforward as initially thought and propose/discuss several potential challenges. If the cross-human-animal comparison, this review will be of interest and useful to those studying neural control of muscle in humans and likewise, will be of interest to those studying mechanisms governing motoneuron excitability in animal models. This review is well written and was enjoyable to read. That being said, I have several comments that I think should be addressed. It is my hope that these comments will strengthen the review.

Comments: Of note, the manuscript was not submitted with line numbers but was generated by the editor upon request. To ensure line number references in this review match that of the document, Page 1 Line 1 starts with ‘Journal of Physiology Topical Review’, Page 2 Line 31: ‘Abstract’, Page 2 Line 47: ‘Introduction’...etc. Page 2 Line 38-40: the statement saying 5HT can have profound effects on force generation during muscle contractions is a bit of an overstatement as the review highlights recent work that shows a very modest change MVC following manipulation of the serotonergic system. While I can agree that 5HT has a potent effect on firing rates, this doesn’t seem to translate to huge changes in maximal muscle force - which is interesting and discussed in the review. I think this discrepancy should either be highlighted in the abstract to provide context for the title of the review. Alternatively, I suggest toning this back statement. Line 73: Why has serotonergic modulation been so extensively studied? While I can agree that it is a potent modulatory system in the control of spinal circuits, it is far from the only one. I think that this would be worth highlighting and would be useful to provide reviews that describe roles for other neuromodulators in the control of spinal circuits/motoneuron excitability (eg. Cholinergic, dopaminergic, noradrenergic, etc.). Line 88: While the effects of ionotropic neurotransmitters may be terminated by removal of the ligand, the effects of neuromodulators, such as 5HT on neuronal function may not necessarily be terminated by reuptake of 5HT given that activation of GPCRs can elicit long lasting changes in ion channel function. This statement should at least be re-written to simply highlight 5HT reuptake, including what is the primary means for 5HT (eg. SERT). Line 125: it is not clear what is meant by evoked responses. Would this be using TMS in humans or in animal models using other approaches? Line 137: worth mentioning is work from Brian Noga’s Lab that used voltammetry to show increases in 5HT that occurs on the time scale of seconds during MLR-evoked fictive locomotion in cats (https://doi.org/10.3389/fncir.2017.00059). Interestingly, 5HT levels begin to increase prior to the onset of fictive locomotion which is supportive of a role for 5HT in setting the network tone for locomotion. Line 146-150: the three mechanisms described would collectively increase neuronal excitability. I think it is worthwhile using precise language to highlight this here. Eg change modulate on Line 150 to increase 152. It is unclear what is exactly meant by ‘inhibition of hyperpolarization’. It is not clear how reducing spike threshold would inhibit hyperpolarization. Lines 152-156 should be rethought and reworded for clarity. Line 160: the DLF is not a specific analogue of the raphe-spinal pathway, but does contain descending serotonergic fibres, in addition to many other. This might be misleading as written. Line 167: which neuron? Line 169: An additional inhibitory and activity dependent mechanism mediated by dynamic sodium potassium ATPase pumps has also been demonstrated by the Sillar lab for control of motoneuron activity in Xenopus tadpoles (Zhang et al. 2012; 2015). Importantly, this inhibitory pump is under the control of 5HT receptors (Hachoumi et al., 2022). Similar inhibitory pump dynamics have also been demonstrated in mammalian motoneurons and can be modulated by dopamine...
relaxation on persistent inward current estimated by two different methods (https://doi.org/10.1113/JP282765)

Exploring the effects of Ia reciprocal inhibition on neuromodulatory commands in the human lower limb

nature, both support the notion that afferent feedback may dampen the effects of PICs in humans. Please refer to the discussion

p8; l235: put "i.e. the motoneurone's hysteresis" in parenthesis?

biceps (near 100%; see pre-fatigue %VA in Fig 3) then we would not expect that they could gain more than a percent or two

of ion channels and possibly (although not known) express different 5HT receptors or have inputs to different regions. Along

of 'PIC-channels' as changes in PIC-mediated intrinsic properties could be mediated through changes in some of these

literature, the threshold of the PIC can vary and may be a key contributor to MN recruitment. Please refer to the discussion

of ion channels and their influence on motoneuron intrinsic properties differ between motoneuron subtypes. This might be a good spot to highlight some of these works (Lee and Heckman 1998, Huh et al. 2017; Sharples and Miles 2021). I think it is also worth highlighting that motoneuron subtypes express different complements of ion channels and possibly (although not known) express different 5HT receptors or have inputs to different regions. Along these lines, studies of different muscles with varying muscle fibre compositions (e.g. Soleus, TA, etc) also might respond differently to neuromodulation. These could possibly contribute to task-differences in function and neuromodulatory control.

Line 253-256: This statement isn't very clear. I suggest breaking it up a bit. Line 256: Not to mention that changes in the expression of the PIC channels or those that oppose their actions could also contribute. Line 313: suggest toning back language from '...in the spinal cord was due to...' to '...in the spinal cord could be due to...' as although plausible, this is purely speculative. Line 348: Modulated 'by' 5HT. Line 369-373: Maybe not such a recent idea with the citations provided approaching 15 years ago. Suggest rephrasing. Line 375: This paragraph ends kind of open. Is there a possibility to capitalise on new genetic tools in rodents that allow for identification and manipulation of defined motoneuron subtypes to advance these ideas further? Are there new methods/technologies/techniques in humans that might provide some insight? Line 377: I'm not entirely sure that this question was answered in this conclusion paragraph. Indeed, challenges are highlighted, but I think it would be worthwhile to outright statement that might provide more of an answer to this question.

Line 378: neuromodulation of what? This sentence is rather vague as 5HT is a neuromodulator and it can be inferred that it would contribute to neuromodulation. I would suggest stating that 5HT is a potent modulator of spinal circuits and motoneuron output. Line 395-396: this sentence is a little misleading as it implies that pharmacological manipulation of neurotransmitters in the CNS is unique to humans. However, I think that the main point is detailed in the sentence that follows. Perhaps consider rephrasing these two sentences. Figure 1: I think this figure is a little problematic as it attempts to synthesize data from multiple species (turtle and mammals). It should be made clearer in the figure itself (in addition to caption) where these data are derived otherwise it is a bit misleading. As highlighted above, it should be highlighted that the DLF contains descending serotonergic fibres and is not simply an analogue of the raphe spinal pathway. Referee #2: It was with pleasure that I read the work submitted by Kavanagh and Taylor entitled *Voluntary activation of muscle in humans: does serotoninergic neuromodulation matter?* For many years, the field has been left wondering if PICs that are facilitated by 5HT are actually relevant to human function. This topical review starts to address this, and other, aspects of 5HT neuromodulation during normal and fatigued motor output. It is with great certainty that I can say that this article will be highly read and cited. Great work! Below, I have provided some specific comments (with page

; and line II] in an attempt to improve the quality of the manuscript: p2; I57: Ionotropic/neuromodulatory is not necessarily a class of input, rather they are inputs that activate classes of receptors. The inputs activate either ionotropic or metabotropic receptors and neuromodulatory inputs would predominantly activate those of the metabotropic class. Slight rewording for clarity would be helpful here. p5; I113: is this suggested to refer to rates of discharge? section ending on I142: The discussion of the role of 5ht on the dorsal cord seems a little light - one might consider a slight expansion of this topic here so that the (predominantly) inhibitory effects of 5ht on sensory transmission can be appreciated. p7; I207-209: It may be nice to put this magnitude of change into perspective for the naive reader. If a healthy young adult has near complete activation of the biceps (near 100%; see pre-fatigue %VA in Fig 3) then we would not expect that they could gain more than a percent or two as they cannot have -complete activation (theoretical, I know). Other muscles, with lower %VA, may show greater change. p8; I235: put "i.e. the motoneurone's hysteresis" in parenthesis? p8; I236-237: Although seldomly considered in the literature, the threshold of the PIC can vary and may be a key contributor to MN recruitment. Please refer to the discussion of Afsharipour et al 2021 Section ending I375: I suggest updating this section with reference to the the emerging findings from both CJ Heckman's lab and from the second author's lab (references below). These findings, albeit preliminary in nature, both support the notion that afferent feedback may dampen the effects of PICs in humans. Pearcey et al. 2020 - Exploring the effects of la reciprocal inhibition on neuromodulatory commands in the human lower limb (https://doi.org/10.1096/fasebj.2020.34.s1.09445) Mesquita et al. 2022 - Effects of reciprocal inhibition and whole-body relaxation on persistent inward current estimated by two different methods (https://doi.org/10.1113/JP282765) Referee #3:
This review provides a comprehensive and balanced overview of the role of 5-HT on muscle activation. Insights from mammalian and from human studies are discussed. The authors do a commendable job of comparing the results from both animal and human studies and identify methodological challenges in testing hypotheses derived from animal work in human studies. The review will be very appreciated by the motor control community. I only have minor comments: 1. I know that this review looks at voluntary muscle activation but I wonder if there are any insights about 5-HT perhaps modulating reflexive movements in humans and whether there could be a greater role in that type of movement based on the possibility that serotonergic systems are activated by novel or surprising stimuli. 2. What about the role of 5-HT in mediating presynaptic inhibition of sensory afferents? Are there insights on this in humans, and does it relate to central fatigue? 3. If I can make a suggestion to add Bui et al. (2003) (work from Ken Rose lab) to the works cited in line 356.

REQUIRED ITEMS:

- Please include an Abstract Figure. The Abstract Figure is a piece of artwork designed to give readers an immediate understanding of the Review Article and should summarise the main conclusions. If possible, the image should be easily ‘readable’ from left to right or top to bottom. It should show the physiological relevance of the Review so readers can assess the importance and content of the article. Abstract Figures should not merely recapitulate other figures in the Review. Please try to keep the diagram as simple as possible and without superfluous information that may distract from the main conclusion of the Review. Abstract Figures must be provided by authors no later than the revised manuscript stage and should be uploaded as a separate file during online submission labelled as File Type 'Abstract Figure'. Please ensure that you include the figure legend in the main article file. All Abstract Figures will be sent to a professional illustrator for redrawing and you may be asked to approve the redrawn figure before your paper is accepted.

- Please upload separate high quality figure files via the submission form.

- Author profile(s) must be uploaded via the submission form. Authors should submit a short biography (no more than 100 words for one author or 150 words in total for two authors) and a portrait photograph of the two leading authors on the paper. These should be uploaded, clearly labelled, with the manuscript submission. Any standard image format for the photograph is acceptable, but the resolution should be at least 300 dpi and preferably more. A group photograph of all authors is also acceptable, providing the biography for the whole group does not exceed 150 words.

END OF COMMENTS
Title: Voluntary activation of muscle in humans: does serotonergic neuromodulation matter?

Authors: Justin Kavanagh
        Janet Taylor

Author Conflict: No competing interests declared

Author Contribution: Justin Kavanagh: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work

Janet Taylor: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work

Running Title: Serotonin and voluntary muscle activation

Dual Publication: Figure 1B, 1C, and 1D: Our schematic of the serotonergic system and motoneurones is supported by cellular data

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published in Cotel, Exley, Cragg, and Perrier JF, Proceedings of the National Academy of Sciences USA, 2013, 110(12):4774-9. This does not constitute dual publication as we use this representative image only to illustrate the main theme of the Topical review. Namely, that prior to our human work, cellular studies had revealed the dual role that serotonin plays in modulating motoneurone activity. The source publication is included in the figure caption. Figures 2 and 3: These are data sets that have been published by the lead author. They are also acknowledged in each figure caption. These figures should not be considered as dual publications as we are only using them to illustrate a series of published experiments which highlights the excitatory effect of serotonin during unfatigued muscle contractions (Figure 2), and the inhibitory effect of serotonin during fatigued muscle contractions (Figure 3). Figures are from: Kavanagh, McFarland and Taylor. Journal of Physiology, 2019, 597, 319-332. Thorstensen, Taylor, Tucker and Kavanagh. Journal of Physiology, 2020, 598, 2685-2701. Thorstensen, Taylor and Kavanagh. Journal of Neurophysiology, 2021, 125, 1279-1288. Henderson, Thorstensen, Morrison, Tucker and Kavanagh. Journal of Neurophysiology, 2022, 127, 27-37.

**Funding:** No funding: Justin J Kavanagh, N/A; No funding: Janet L Taylor, N/A

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Voluntary activation of muscle in humans: does serotonergic neuromodulation matter?

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Running title: Serotonin and voluntary muscle activation

Keywords: Serotonin; Neuromodulation; Motoneurones; Persistent inward current; Fatigue.

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Abstract

Ionotropic inputs to motoneurones have the capacity to depolarise and hyperpolarise the motoneurone, whereas neuromodulatory inputs control the state of excitability of the motoneurone. Intracellular recordings of motoneurones from in vitro and in situ animal preparations have provided extraordinary insight into the mechanisms that underpin how neuromodulators regulate neuronal excitability. However, far fewer studies have attempted to translate the findings from cellular and molecular studies into a human model. In this review, we focus on the role that serotonin plays in muscle activation in humans. Serotonin (5-HT) is a potent regulator of neuronal firing rates and can have profound effects on the amount of force that can be generated by muscles during voluntary contractions. We firstly outline structural and functional characteristics of the serotonergic system, and then describe how motoneurone discharge can be facilitated and suppressed depending on the 5-HT receptor subtype that is activated. We then provide a narrative on how 5-HT effects can influence voluntary activation during muscle contractions in humans, and detail how 5-HT may be a mediator of exercise-induced fatigue that arises from the central nervous system.

Introduction

The final common pathway in the motor system is the motor unit, which consists of the alpha motoneurone in the spinal cord and all muscle fibres that it innervates. Given that the motor unit links the central nervous system to skeletal muscle, efficient and purposeful movement can only be achieved by tightly controlled motoneurone activity. The firing pattern of a motoneurone is produced by converting synaptic inputs into action potentials, where the regulation of input to output is determined by the intrinsic electrical properties of motoneurons (Kernell, 2006). Hence, to gain a better understanding of how the nervous system regulates motor activity, it is of great importance to explore how inputs to the motoneurone influence activation of muscle.

There are two classes of inputs to motoneurones: ionotropic and neuromodulatory. In a general sense, ionotropic inputs are command signals from descending motor pathways, peripheral afferents, or spinal interneurons, that have the capacity to depolarise and hyperpolarise the motoneurone. Typically, ionotropic receptors are ligand-gated ion channels which directly gate ion flow into cells to generate either an excitatory or inhibitory response in postsynaptic neurons (Eccles & McGeer, 1979; Heckman & Binder, 1991; Powers et al.,
In contrast, neuromodulatory inputs control the state of excitability of the motoneurone through intracellular signalling pathways that modify the motoneurone’s responsiveness to ionotropic input (Eccles & McGeer, 1979; Elliott & Wallis, 1992; Fedirchuk & Dai, 2004; Heckman et al., 2008a; Murray et al., 2011). The role that neuromodulators play in activating motoneurones has received considerable attention in the past 30 years. Most notably, intracellular recordings of motoneurones from *in vitro* and *in situ* animal preparations have provided considerable insight into how electrical properties of motoneurones are influenced by neuromodulators. However, fewer studies have attempted to translate findings from intracellular experiments into humans, as there are considerable challenges in reproducing reductionist experiments in an intact human neuromuscular system.

This topical review will survey how serotonergic neuromodulation contributes to voluntary activation of muscle in humans. To achieve this, the functional anatomy of serotonergic pathways with regard to the motor system will be outlined before summarising human experiments that assess the role of this neuromodulator in performing unfatigued and fatiguing muscle contractions.

The serotonergic system

Serotonin (or 5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter that modulates the intrinsic properties of neurons in the central nervous system (CNS). In the periphery, it is synthesised in enteric mucosal cells, influences gut motility among other functions, and is stored in platelets (Terry & Margolis, 2017). However, 5-HT is unable to cross the blood-brain barrier and must be synthesised within the CNS from its precursor, tryptophan. Hence, the primary site of CNS 5-HT synthesis in vertebrates is the raphe nuclei of the brainstem (Hery *et al.*, 1982; Imai *et al.*, 1986; Hornung, 2003; Pollak Dorocic *et al.*, 2014). At a cellular level, 5-HT is released into synapses and exerts pre- and post-synaptic effects when it binds to membrane receptors. The termination of 5-HT effects at the synapse occurs with reuptake into pre-synaptic terminals or glial cells via monoamine transporters. Seven classes of 5-HT receptors have been identified (Lucas & Hen, 1995). They are all G-protein coupled receptors (Hoyer & Martin, 1997) except for the 5-HT₃ receptor which is a ligand-activated channel (Thompson & Lummis, 2006). However, the principal 5-HT receptors expressed by motoneurones are 5-HT₁₆, 5-HT₁₈, 5-HT₂₆, 5-HT₂₈, 5-HT₂₆. For comprehensive reviews on
5-HT receptor subtypes in the CNS refer to (Peroutka, 1990; Barnes & Sharp, 1999; Nichols & Nichols, 2008; Perrier et al., 2013).

From the brainstem, neurons from a group of serotonergic nuclei ascend to distribute across many areas of the brain, including the frontal lobe where 5-HT can be released in the cerebral cortex (Figure 1A). A high density of 5-HT fibres have been identified in motor areas of the rat and primate cortex (Vertes, 1991; Wilson & Molliver, 1991), however the role of these rostral raphe projections in motor activity has not been well-defined. Cortical structures predominantly express receptors from the inhibitory 5-HT$_{1A}$ family and to a lesser extent the excitatory 5-HT$_{2A}$ family (Pazos et al., 1985; Pazos & Palacios, 1985; Celada et al., 2013), where 5-HT$_{1A}$ is expressed extensively in laminae II/III of the motor cortex and 5-HT$_{2A}$ receptors are uniformly distributed across motor cortex laminae (Joyce et al., 1993; Vitrac & Benoit-Marand, 2017). It appears that depleting brain 5-HT in rats has the effect of reducing the excitability of neural circuits in motor cortex (Scullion et al., 2013), and administration of selective serotonin reuptake inhibitors (SSRIs) enhances excitability in motor cortex circuits in humans (Ilic et al., 2002; Gerdelat-Mas et al., 2005). Thus, a likely key role of 5-HT in the motor cortex is modulation of facilitatory intracortical motor circuits. 5-HT also appears to play a critical role in plasticity of motor circuits, as SSRI administration combined with either non-invasive brain stimulation or motor practice can influence excitatory synaptic plasticity. In particular, there is evidence to suggest enhanced long-term potentiation-like plasticity from paired associative stimulation (Nitsche et al., 2009; Batsikadze et al., 2013), whereas changes in plasticity from practicing motor tasks has been observed in most (Classen et al., 1998; Loubinoux et al., 2002; Pleger et al., 2004) but not all (McDonnell et al., 2018) experiments that have assessed cortical plasticity after SSRI ingestion. Functional magnetic resonance imaging has identified several interconnected structures that are affected by enhanced 5-HT concentration, where short-term drug-induced neuroplastic changes are associated with enhanced activation of primary motor and premotor cortices, as well as posterior supplementary motor areas (i.e. executive motor areas of the cortex) (Loubinoux et al., 1999).

< insert Figure 1 >

Most of the work that has examined 5-HT effects on motor cortical activity has focused on evoked responses and has not linked cortical changes to muscle performance. However,
considerably more is known about how 5-HT release on motor circuits in the spinal cord directly affects motor activity. From the brainstem, the raphe-spinal pathway descends to the spinal cord to form well-defined synapses with motoneurones, afferent neurons, and interneurons (Figure 1A) (Ridet et al., 1994; Alvarez et al., 1998; Kawashima, 2018). There are approximately 1500 serotoninergic contacts on each motoneurone (Alvarez et al., 1998), and the intensity of 5-HT release from the raphe-spinal pathway, but not the ascending pathway, is thought to correspond to the intensity of motor activity being performed by upper limb (forelimb) and lower limb (hindlimb) muscles. This viewpoint has predominantly been based on cat studies that show activity in the raphe nuclei is abolished during sleep (McGinty & Harper, 1976; Cespuglio et al., 1981), but near-linearly scaled to locomotion speed (Veasey et al., 1995; Jacobs & Fornal, 1997; Jacobs et al., 2002). Incremental increases in treadmill walking speed correspond to incremental increases in single raphe-spinal fibres (Fornal et al., 2006), which potentially points towards a contraction intensity dependent mechanism of 5-HT release. On face value, this suggests a very useful motor control strategy, in which strong contractions cause large release of 5-HT to amplify signals from synaptic inputs. However, a phenomenon exists where the presence of too much 5-HT in the CNS can limit the ability of motoneurons to activate muscle.

**Dual role of 5-HT on motoneurone activity**

There is widespread agreement that 5-HT promotes depolarisation in motoneurones, which arises from several concomitant mechanisms. In particular, cellular and molecular experiments have identified that 5-HT can facilitate a rectifying inward current (Takahashi & Berger, 1990; Hsiao et al., 1997), facilitate low threshold Ca$^+$ currents (Berger & Takahashi, 1990), or inhibit K$^+$ leak conductance (Elliott & Wallis, 1992; Perrier et al., 2003) to modulate neuronal excitability. 5-HT can also increase discharge rate via inhibition of slow and medium afterhyperpolarisation (Hounsgaard et al., 1988b; Hounsgaard & Kiehn, 1989; Bayliss et al., 1995; Wikstrom et al., 1995; Grunnet et al., 2004). Although 5-HT can inhibit hyperpolarisation by decreasing the threshold for generating a Na$^+$ based action potentials (Fedirchuk & Dai, 2004), 5-HT-mediated inhibition of hyperpolarisation also generates a Ca$^+$ dependent plateau potential by reducing a K$^+$ current responsible for slow afterhyperpolarisation (Hounsgaard & Kiehn, 1989). In general, the functional role of 5-HT on motoneurone activity has been revealed by slice preparations, partially intact animal preparations, and simulations. With these approaches, it has been possible to examine the
interaction between physiological 5-HT concentrations and 5-HT receptor subtypes. Electrical stimulation of the dorsolateral funiculus (DLF, analogous to the raphe-spinal pathway) in integrated preparations of the adult turtle spinal cord has been shown to promote the release of endogenous 5-HT into the ventral horn of the spinal cord (Delgado-Lezama et al., 1997; Perrier & Delgado-Lezama, 2005; Perrier & Cotel, 2008b). In this circumstance, motoneurone recordings have confirmed that discharge rate increases with brief bursts of 5-HT release (Figure 1B) (Hounsgaard & Kiehn, 1989; Perrier & Hounsgaard, 2003; Perrier & Delgado-Lezama, 2005), and this increase is mediated by activation of 5-HT$_{2B/C}$ receptors located on the dendrites and the cell body of the neuron (Jackson & White, 1990; Cotel et al., 2013).

In addition to 5-HT exerting excitatory effects on motoneurones, the presence of this neuromodulator has also been observed to create an inhibitory effect on motor activity. Early in vivo work that recorded motoneurone activity in cats observed that 5-HT injected in the vicinity of the motoneurone increased hyperpolarisation, which caused a failure in the motoneurone to fire (Phillis et al., 1968). An underlying mechanism of 5-HT-mediated inhibition of motoneurone activity was clarified in a series of elegant experiments by Perrier and colleagues. Using preparations from the turtle spinal cord, it was revealed that the ability of motoneurones to fire is inhibited when 5-HT is iontophoretically applied close to motoneurone soma (Perrier & Hounsgaard, 2003) and when prolonged DLF stimulation causes large endogenous release of 5-HT onto motoneurones (Figure 1C) (Cotel et al., 2013; Perrier et al., 2017). The mechanism underlying 5-HT’s dual excitatory and inhibitory effects aligns with the compartmentalisation of 5-HT receptor subtypes expressed on motoneurones. The monosynaptic connections that raphe-spinal neurons have with motoneurones causes direct 5-HT release onto receptors in somatodendritic regions to enhance motoneurone excitability. However, activation of 5-HT$_{1A}$ receptors expressed in perisomatic regions inhibits Na$^+$ channels responsible for the genesis of action potentials, which has the effect of preventing motoneurone firing (Perrier & Cotel, 2008a). Given that the inhibitory 5-HT$_{1A}$ receptors are only located on the axon initial segment, and the axon initial segment is devoid of serotonergic innervation, activation of 5-HT$_{1A}$ receptors can only occur if extracellular concentrations of 5-HT are large enough to spill over onto the axon initial segment (Cotel et al., 2013; Perrier & Cotel, 2015; Perrier et al., 2017). These findings provide a potential cellular mechanism for central fatigue and provide a foundation to investigate if 5-HT activity during prolonged bouts of physical activity reduces motor output in humans.
Is serotonergic neuromodulation reflected in voluntary muscle activation in humans

Although it is difficult to identify how individual neuromodulators contribute to motor activity, simulations indicate that the net effect of maximal neuromodulation may be a three-to five-fold amplification of the currents that the motoneurons receive from synaptic inputs (Lee & Heckman, 2000; Hultborn et al., 2003). Hence, the availability of 5-HT is critical to enhancing the gain of motoneurone output, and ultimately, activating the muscle to produce force. Data from healthy adults performing maximal effort contractions mostly support this finding. However, the magnitude of response in human experiments is small (Figure 2A and 2B). Following the administration of the SSRI paroxetine to increase serotonin availability, the force of maximal voluntary contractions (MVC) has been noted to increase 1.5-4.5% in several experiments employing elbow flexion protocols. Similarly, voluntary activation increased by 0.3% when measured using cortical stimulation (Thorstensen et al., 2020) and by ~1.3% when measured using motor nerve stimulation (Kavanagh et al., 2019; Thorstensen et al., 2020). A finding of only small drug-related increases in voluntary activation is perhaps not surprising, as increases in motoneurone excitation during near-maximal contraction intensities produce only small changes in interpolated twitch amplitude (which is a key variable in calculating voluntary activation) (Herbert & Gandevia, 1999). Nevertheless, an enhanced ability to activate the muscle is consistent with 5-HT effects that are mediated by excitatory somato-dendritic 5-HT$_2$ receptors on motoneurones. Consistent with this, the administration of a 5-HT$_2$ competitive antagonist leads to the opposite effect on maximal force generation. Maximal elbow flexion force declined by ~3% after the administration of cyproheptadine (Thorstensen et al., 2021). Antagonism of the 5-HT$_2$ receptor also leads to a reduction in rate of force development when performing rapid dorsiflexions, which is mediated by reduced firing rates in motor units of the tibialis anterior (Goodlich et al., 2022). Thus, enhancing 5-HT availability during strong unfatigued contractions increases force generation, whereas blocking 5-HT$_2$ receptor activity decreases force generation via neural mechanisms in healthy individuals.
Persistent inward currents (PIC) play a critical role in setting the gain of motoneurones. PICs are mediated by voltage-gated sodium channels (Na\textsubscript{v}1.1 and Na\textsubscript{v}1.6) and L-type voltage-gated calcium channels (Ca\textsubscript{v}1.2 and Ca\textsubscript{v}1.3) on somato-dendritic surfaces of motoneurones (Schwindt & Crill, 1977; Schwindt & Crill, 1980; Heckmann et al., 2005). In mammals, there is believed to be equal contributions from slow activating L-type Ca\textsuperscript{2+} current and a fast activating persistent Na\textsuperscript{+} current to the PIC (Heckman et al., 2008a). Moreover, PICs are strongly influenced by monoamines. Activation of PICs causes a remarkable amplification of depolarising drive to the motoneurone and evokes a strong acceleration of motoneurone firing rate (Hounsgaard et al., 1988a; Bennett et al., 1998; Lee & Heckman, 1998). Thus, it might be expected that PICs are responsible for 5-HT mediated increases in voluntary activation during MVCs. In humans, the amplitude of PIC activation can only be estimated. The accepted technique for this estimation for an individual motoneurone compares the estimated synaptic input that recruits a motoneurone to that at its derecruitment i.e. the motoneurone’s hysteresis. PICs provide little drive to the motoneurone at recruitment as initiation of an action potential and the activation of PICs occur at a similar membrane potential. By comparison, PICs are fully active after the motoneurone has been firing for ~ 0.5-1.0 s (Lee & Heckman, 1996; Heckman et al., 2008b). Thus, at derecruitment, PICs contribute part of the depolarising current for motoneurone firing so that less synaptic input is required.

The paired motor unit technique uses the firing rate of low threshold motor units, that already have PICs fully active, as estimates of synaptic input to other motor units in the same pool. The measure that is calculated is known as ΔF (difference in frequency of firing of the lower threshold motor unit at recruitment and derecruitment of the higher threshold motor unit). Discharge rates are recorded from intramuscular electromyography electrodes (Gorassini et al., 2002; Udina et al., 2010; Foley & Kalmar, 2019) or extracted from high-density electromyography arrays placed over the muscle (Afsharipour et al., 2020; Hassan et al., 2020). The idea that the influence of serotonin on human motor performance is mediated via PICs is supported by pharmacological studies in a small number of healthy participants (n = 3), in whom ΔF increased with ingestion of an SSRI or decreased with a 5-HT\textsubscript{2} receptor antagonist (D’Amico et al., 2013). Nonetheless, because ΔF has only been calculated during submaximal ramped-shaped contractions, it is unknown if PICs are directly linked to enhanced MVCs in humans. However, it is known that PICs associated with soleus and gastrocnemius medialis increase from 10% to 20% MVC with additional increases to 30% MVC for soleus, which suggests that PIC activation is linked to voluntary drive and hence 5-
HT release (Orssatto et al., 2021b). Reductions in motoneurone PIC may also provide a mechanism for loss of force production from skeletal muscle. In younger populations, there is evidence that ΔF reduces up to 25% for soleus motoneurones following passive stretching of the plantarflexors, which may explain some of the reductions in force associated with stretching protocols (Trajano et al., 2020). In older populations, ΔF is lower than in young adults for soleus and tibialis anterior (Orssatto et al., 2021a), and biceps and triceps brachii (Hassan et al., 2021), and may contribute to age-related reductions in motoneurone firing rates and muscle force. However, the mechanisms underlying the reductions in PIC activity are not known. Conceivably, monoamine release onto the motoneurones could be reduced or the motoneuronal response to monoamines could be altered by changes in receptors. Alternatively, PICs are sensitive to inhibitory synaptic input and could be turned off by added inhibition.

Is prolonged release of serotonin a mechanism of fatigue in humans?

Serotonin has long been implicated in the development of fatigue in humans, where the performance of prolonged exercise increases blood tryptophan levels and thus 5-HT synthesis in the CNS (Newsholme et al., 1987). The ‘central fatigue hypothesis’ was introduced in the 1980s, when it was proposed that enhanced concentration of brain 5-HT induced negative effects on arousal and mood, and increased lethargy and sleepiness. It was postulated that this mechanism could influence the perception of effort and, therefore, fatigue (Newsholme et al., 1987; Newsholme & Blomstrand, 2006). Following this original hypothesis, several studies used pharmacological interventions to modify CNS 5-HT levels during prolonged cycling, and found that cycling performance was sometimes (Meeusen et al., 2001; Strachan et al., 2004; Roelands et al., 2009) but not always (Wilson & Maughan, 1992; Strüder et al., 1998; Teixeira-Coelho et al., 2014) limited by enhanced 5-HT concentrations. These mixed findings are most likely a reflection on the complexity of neurotransmitter systems, and no single neurotransmitter is responsible for exercise-induced central fatigue. Indeed, pharmacological interventions that antagonise D2 dopaminergic receptors (Thorstensen et al., 2018) and inhibit noradrenaline reuptake (Klass et al., 2012; Klass et al., 2016) have been shown to reduce voluntary muscle activation during fatiguing contractions in humans. Another difficulty with interpretation of pharmacological interventions during whole-body exercise is that widespread, and often contradictory interactions of serotonin occur for the motor system. Even at a supraspinal level, if increased cortical 5-HT does reduce motivation
and increase effort through actions at high level motor areas, increased motor cortex excitability with SSRI ingestion suggests that corticospinal pathways may be facilitated (Ilic et al., 2002; Gerdelat-Mas et al., 2005).

Benchtop experiments cannot replicate the same physiological conditions associated with exercise-induced fatigue. However, recordings obtained from animal preparations strongly suggest that activation of 5-HT\textsubscript{1A} receptors in the spinal cord would limit the ability for the CNS to activate muscle, and thus contribute to central fatigue. This specific mechanism appears relevant to humans, as ingestion of the 5-HT\textsubscript{1A} receptor agonist, buspirone, suppresses spinal motoneurone excitability (D’Amico et al., 2017) and reduces the capacity to perform prolonged bouts of exercise in healthy individuals (Marvin et al., 1997). However, exogenous activation of the 5-HT\textsubscript{1A} receptor may cause different responses to naturally occurring 5-HT dynamics, so we performed several experiments to assess muscle activation when endogenously released 5-HT is accumulated in the CNS. Fatiguing prolonged repeated maximal effort contractions were performed with and without enhanced availability of 5-HT caused by ingestion of an SSRI. We observed that MVC torque and time-to-task failure were reduced when more 5-HT was available (Figure 3A) (Kavanagh et al., 2019). That is, more fatigue developed more quickly. It was evident that this decline in performance had neural origins, as fatigue-related failure of voluntary activation was greater in the presence of higher 5-HT concentrations (Figure 3B). Additional experiments were performed using the hand muscle abductor digiti minimi, where fatigue-related reductions in F-wave persistence and F-wave area following a prolonged MVC were substantially greater with greater 5-HT availability. As F waves are a marker of motoneurone excitability, these findings indicated that reduced excitability of spinal motoneurones contributed to the added central fatigue caused by 5-HT reuptake inhibition. Consistent with the idea of serotonin spill over as described above, it is likely that the SSRI accumulation of 5-HT in the spinal cord was due to strong serotonergic drive occurring when neural drive to the target muscle was high. The high levels of synaptic serotonin could then spill over to activate the extra-synaptic inhibitory 5-HT\textsubscript{1A} receptors on motoneurones to suppress voluntary muscle activation.

< insert Figure 3 >
Prolonged low-intensity contractions can also lead to substantial declines in motor performance due to central fatigue (Sogaard et al., 2006). During the performance of a 15% MVC for 30 min, the development of muscle fatigue was demonstrated by a reduction in force of occasional brief MVCs, and central fatigue by associated declines in voluntary activation. By comparing performance with and without serotonin reuptake inhibition, we were able to test if 5-HT effects progressively limit muscle activation over time as the sustained contraction presumably causes a sustained release of 5-HT in the CNS (Thorstensen et al., 2020). Contrary to our hypothesis, muscle force, voluntary activation, and corticospinal excitability were not affected by 5-HT reuptake inhibition during the prolonged low-intensity contraction (Figure 3C and 3D). The absence of 5-HT effects may have occurred due to two possible mechanisms. First, during prolonged low-intensity contraction, neural drive to the muscle may not have been of a sufficient intensity to cause intense release of 5-HT from the raphe-spinal pathway onto motoneurons. Hence, serotonin reuptake may have kept up with serotonin release despite the SSRI. Second, serotonergic drive may have declined throughout the contraction and caused a reduction of 5-HT release onto motoneurons. The latter mechanism has support from cat experiments as the firing of raphe-spinal neurons are reported to progressively decrease during sustained physical activity. In single raphe-spinal neurons activity decreased by up to 50% after 40 min of treadmill walking (Fornal et al., 2006). Thus, serotonergic neuromodulation may not be an endless resource during muscle contractions. As yet, it is not clear what level and duration of voluntary contraction is enough to elicit fatigue-related motoneurone inhibition through serotonin spill over. Our experiments suggest that 30 min of a sustained 15% MVC is insufficient, but 40-60 s of a sustained MVC is sufficient.

Afferent feedback can potentially regulate 5-HT effects at the motoneurone

Voluntary activation of muscle can be modulated by muscle afferent feedback. This presents a challenge for understanding motoneurone excitability, as ionotropic input received by the motoneuron comprises concurrent excitatory (descending drive and muscle spindle afferents) and inhibitory (group Ib, group III and IV) synaptic activity that can be modulated with 5-HT (Figure 1). Adding to this complexity, most muscle afferents synapse on interneurons in the spinal cord to cause excitation and/or inhibition in multiple target muscles. Thus, examining the action of individual afferents, and recording activity from individual muscles, may not reflect the net output of the motor system (D'Amico et al., 2014).
Although the amplitude of dendritic PICs is directly proportional to the intensity of brainstem neuromodulatory drive, inhibition from low-threshold sensory inputs has a strong suppressive effect on PIC amplitude. When inhibition is strong enough, the PIC created by the monoaminergic drive are reduced or even deactivated (Hultborn et al., 2003; Kuo et al., 2003). *In vivo* cat experiments have revealed that reciprocal inhibition may be especially critical for regulating PIC amplitude during functional motor activity. Even minor rotations of the ankle joint that cause barely detectable changes in reciprocal inhibition of motoneurones will reduce PICs by ~50% (Hyngstrom et al., 2007). The likely candidate for this afferent mechanism is Ia disynaptic reciprocal inhibition evoked by length changes in the antagonist muscle, as other afferents are relatively insensitive to the changes in muscle length that occurred with passive rotation of the cat ankle in the experiment (Hyngstrom et al., 2007). Interestingly, reciprocal inhibition may solve a motor control problem that arises from the diffuse projection of monoaminergic fibres in the spinal cord. Widespread projections from the serotonergic system will release 5-HT on more than one motor pool during voluntary contractions. Hence, 5-HT may simultaneously enhance PIC activity on multiple motoneurones, which has the potential to create a bias towards co-contraction due to increasing excitability in antagonist motor pools (Heckman et al., 2008a). A recent proposal suggests that Ia reciprocal inhibition may promote voluntary activation during a variety of motor tasks by only enhancing activity in task specific muscles from a background of diffuse excitatory neuromodulation (Hyngstrom et al., 2007; Heckman et al., 2008a; Hyngstrom et al., 2008). However, as already intimated, translating the findings of animal experiments to purposeful motor activity in humans is challenging, and interpretations such as these must be further explored.

**So does serotonergic neuromodulation matter in humans?**

Animal experiments provide clear evidence that 5-HT contributes to neuromodulation. However, the effects of 5-HT on voluntary muscle activity in humans are less clear. This is not to say that serotonergic neuromodulation does not matter for humans, but instead highlights the challenges associated with studying how a complex neuromodulatory system acts during muscle contractions. Controlled experiments using single-joint, single muscle, contraction protocols have found that maximal force can be changed by altering 5-HT activity in the CNS. However, the differences observed on voluntary activation are small. Nonetheless, 5-HT-related changes in muscle activation typically emerge with strong
contractions for both the unfatigued and fatigued motor system. Thus, it appears that the magnitude of descending drive to the muscle may be aligned with the level of 5-HT neuromodulation in humans. Indeed, we are beginning to reveal evidence where 5-HT-effects may be scaled to the intensity of muscle activation in humans (Goodlich et al., 2022; Henderson et al., 2022).

An inability to quantify 5-HT release onto motoneurones, as well as quantifying the binding affinity to different 5-HT receptor subtypes, presents a challenge for interpreting any neuromodulation experiment (not just human studies). However, a challenge that is unique to human experiments is the use of pharmacology to manipulate neurotransmitter activity in the CNS. Human studies operate within a window of ‘patient safety’, and typically use therapeutic doses of 5-HT modulating drugs. Thus, very little is known about how dosage effects influence 5-HT activity in humans. Pharmacological interventions are also non-specific, and will not have 100% effectiveness when competitively agonising, antagonising, or inhibiting 5-HT reuptake in humans. Individual variation in pharmacokinetics and drug responses also pose a challenge when studying humans, however variability in 5-HT responses is also a prevalent feature of in vitro and in vivo animal experiments that examine motoneurone and afferent activity. It is our position that none of these factors should prevent human 5-HT research from continuing, but instead should encourage scientists to further explore the relationship between neuromodulation and voluntary muscle activation.

Competing interests and funding

The authors declare that no competing interests exist for this work and no funding was received to perform this work.

Author contributions

Both authors contributed to the conception and the design of this work, as well as the drafting and final approval of the manuscript.
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Figure 1. Serotonergic activity modulates cortical and spinal motoneurone activity.

(A) Serotonin synthesis in the mammalian CNS occurs in the raphe complex of the brainstem. Rostral raphe nuclei ascend primarily to the forebrain to innervate virtually all regions. Caudal raphe nuclei project mainly to the cerebellum and spinal cord, where the monosynaptic raphe-spinal pathway releases 5-HT on the soma and dendrites of spinal motoneurones. Ionotropic inputs from descending motor cortical pathways, peripheral afferents, or spinal interneurons, have the capacity to depolarise and hyperpolarise the motoneurone. However, activation of 5-HT receptors, predominantly in the 5-HT$_1$ and 5-HT$_2$ families, regulate the state of excitability of the motoneurone via intracellular signalling pathways to modify the motoneurone’s responsiveness to ionotopic input. (B) Intracellular recordings from motoneurones in an adult turtle spinal cord preparation (black). The number of action potentials evoked by depolarizing current pulses injected in
the soma is increased following 1 s of 40 Hz electrical stimulation of the dorsolateral funiculus (red) which is analogous to the 5-HT releasing raphe-spinal pathway. (C) Lengthening the duration to 3 min of 40 Hz electrical stimulation inhibited motoneurone firing compared to the control, which is attributed to 5-HT spill over onto extracellular 5-HT$_{1A}$ receptors on the axon initial segment. (D) 3 min of 40 Hz stimulation in the presence of the 5-HT$_{1A}$ antagonist WAY-100635 (20 µM) removed the inhibitory influence of 5-HT. Data sourced from (Cotel et al., 2013)
Figure 2. Elbow flexion MVC torque after ingestion of paroxetine or cyproheptadine. 

(A) The SSRI paroxetine (Parox) exerts small, but significant, increases in torque during brief unfatigued MVCs (paired t-test, \( p = 0.046 \)). Given that voluntary activation of biceps is high in healthy individuals (e.g. 97-99%), a ceiling effect exists whereby only small increases in voluntary activation and elbow flexion torque is possible. (B) Competitive antagonism of the 5-HT\(_2\) receptor via cyproheptadine (Cypro) causes significant reductions in the ability to generate elbow flexion torque during brief unfatigued MVCs (paired t-test, \( p = 0.003 \)). Data are sourced from a combination of studies from the host laboratory using healthy adults aged 20-30 yr (Kavanagh et al., 2019; Thorstensen et al., 2020; Thorstensen et al., 2021; Henderson et al., 2022).
Figure 3. The effect of enhanced availability of 5-HT on maximal and submaximal fatiguing isometric contractions. Paroxetine (Parox) was used to enhance 5-HT concentrations by reuptake inhibition. Motor nerve stimulation was used to assess voluntary activation of the elbow flexors. (A) Time-to-task failure was assessed for 4 sustained maximal elbow flexions, where each contraction was maintained until force declined to 60% MVC. Grey bars indicate a fatiguing contraction has occurred. A rest period of 40 s occurred between maximal contractions. (B) Each fatiguing contraction was followed 3 s later by a resting twitch, and then 3 s later by a brief maximal contraction with superimposed twitch, where voluntary activation was calculated as \[1 - \text{(superimposed twitch/resting twitch)}\] x 100. Enhanced 5-HT concentration caused a significantly greater fatigue-related reduction in time-to-task failure (p = 0.038) and voluntary activation (p = 0.048). (C) Elbow flexion torque has also been assessed during a sustained 15% MVC, where the contraction was held for 30 min and fatigue responses were continued to be monitored for 10 min of recovery. (D) Superimposed and resting twitches were obtained periodically throughout the contraction protocol and during recovery to calculate voluntary activation. There were no changes to fatigue-related reductions in MVC torque (p = 0.376) and voluntary activation (p = 0.505) due to enhanced 5-HT availability. Data sourced from (Kavanagh et al., 2019) and (Thorstensen et al., 2020).
REVIEWING EDITOR

This manuscript has been reviewed by 3 expert reviewers. They unanimously enjoyed reading this review, noted its scholarship qualities and ultimately recommend publication. Several minor suggestions and clarifications are proposed and they appear to be expert and reasonable. As such, the authors should have no difficulties in addressing them in a timely manner.

We thank the Reviewing Editor for the positive comments regarding our manuscript. We have addressed each referee in turn and have highlighted in our manuscript where amendments have been made.

REFEREE #1

Kavanagh and Taylor provide a timely review with the goal of highlighting and summarizing recent work in humans that describe roles and underlying mechanisms for 5HT and target receptors in the voluntary activation of muscle in humans. To accomplish this end, they place their human work in the context of multiple animal models that describe how serotonin modulates intrinsic properties and ion channels of motoneurons. The review draws the conclusions that studying 5HT control of muscle activation in humans might not be as straightforward as initially thought and propose/discuss several potential challenges. Because of the cross-human-animal comparison, this review will be of interest and useful to those studying neural control of muscle in humans and likewise, will be of interest to those studying mechanisms governing motoneuron excitability in animal models. This review is well written and was enjoyable to read. That being said, I have several comments that I think should be addressed. It is my hope that these comments will strengthen the review.

We thank Referee #1 for these positive comments. The referee has provided exceptional comments for us to consider, and we have incorporated nearly all suggestions in our revised manuscript. We would, however, like to preface our responses by pointing out that the majority of recommendations were for the animal and cellular sections of the manuscript. We had to carefully consider how much additional information to include in these sections so our Topical Review retains its focus on human muscle activation.

Of note, the manuscript was not submitted with line numbers but was generated by the editor upon request. To ensure line number references in this review match that of the document, Page 1 Line 1 starts with 'Journal of Physiology Topical Review', Page 2 Line 31: 'Abstract', Page 2 Line 47: 'Introduction'...etc.

We have included line numbers in our revised manuscript.

Page 2 Line 38-40: the statement saying 5HT can have profound effects on force generation during muscle contractions is a bit of an overstatement as the review highlights recent work that shows a very modest change MVC following manipulation of the serotonergic system. While I can agree that 5HT has a potent effect on firing rates, this doesn't seem to translate to huge changes in maximal muscle force - which is
interesting and discussed in the review. I think this discrepancy should either be highlighted in the abstract to provide context for the title of the review. Alternatively, I suggest toning back this statement.

To keep the abstract succinct, we have decided to tone back this statement. We feel that drawing attention to this particular discrepancy may detract from the many other comparisons that are made throughout the review. Our statement now reads, “Serotonin (5-HT) is a potent regulator of neuronal firing rates which can influence the force that can be generated by muscles during voluntary contractions.

Line 73: Why has serotonergic modulation been so extensively studied? While I can agree that it is a potent modulatory system in the control of spinal circuits, it is far from the only one. I think that this would be worth highlighting and would be useful to provide reviews that describe roles for other neuromodulators in the control of spinal circuits/motoneuron excitability (e.g. Cholinergic, dopaminergic, noradrenergic, etc.).

We agree that several neuromodulator systems are involved in the regulation of motor circuits in the CNS. We have included the following information in the revised introduction with a view of remaining focussed on the serotonergic system, but also acknowledging that other neurotransmitter systems in the CNS contribute to muscle activation:

“There are several neuromodulator systems that regulate the excitability of cortical and spinal motor circuits, and many excellent reviews have been written that describe the effects of each system on motor activity (e.g. the cholinergic system (Jones, 2008; Deffains & Bergman, 2015; Naicker et al., 2017; Mille et al., 2021), the dopaminergic system (Sharples et al., 2014; Ikeda et al., 2015; Klaus et al., 2019; Arber & Costa, 2022), the noradrenergic system (Fung et al., 1994; Balaban, 2002; Benarroch, 2018)). Our topical review will provide a unique summary regarding how serotonergic neuromodulation contributes to voluntary activation of muscle in humans.”

Line 88: While the effects of ionotropic neurotransmitters may be terminated by removal of the ligand, the effects of neuromodulators, such as 5HT on neuronal function may not necessarily be terminated by reuptake of 5HT given that activation of GPCRs can elicit long lasting changes in ion channel function.

We agree that this is an important consideration that should be included in the manuscript. We have now modified our text to state:

“It is often suggested that the termination of 5-HT effects at the synapse occurs with reuptake into pre-synaptic terminals or glial cells via monoamine transporters. However, serotonergic effects on neuronal function may not necessarily end with reuptake of 5-HT, as activation of G protein-coupled receptors can elicit long lasting changes in ion channel function (Pavlos & Friedman, 2017)”.

Line 125: it is not clear what is meant by evoked responses. Would this be using TMS in humans or in animal models using other approaches?
This was a poorly worded sentence, and we have changed the text to read, “Most of the work that has examined 5-HT effects on motor cortical activity has employed magnetic or electrical stimulation techniques to explore cortical plasticity, or neuroimaging techniques to map changes in regional activity due to changes in neurotransmitter concentration.”

**Line 137:** worth mentioning is work from Brian Noga's Lab that used voltammetry to show increases in 5HT that occurs on the time scale of seconds during MLR-evoked fictive locomotion in cats ([https://doi.org/10.3389/fncir.2017.00059](https://doi.org/10.3389/fncir.2017.00059)). Interestingly, 5HT levels begin to increase prior to the onset of fictive locomotion which is supportive of a role for 5HT in setting the network tone for locomotion.

We thank Reviewer 1 for highlighting this work, as it has provided a valuable piece of information for our manuscript. Although the reviewer has suggested that this study could be included in our discussion of contraction intensity, we believe that it is best suited several sentences earlier where the onset of 5-HT release complements our anatomical descriptions of the 5-HT system. Our revised sentence now reads, “There are approximately 1500 serotoninergic contacts on each motoneurone (Alvarez et al., 1998), where the release of 5-HT occurs 0.5-1.0 s following the onset of detectable neural activation of locomotor muscles (Noga et al., 2017)”.

**Line 146-150:** the three mechanisms described would collectively increase neuronal excitability. I think it is worthwhile using precise language to highlight this here. Eg change modulate on Line 150 to increase.

We have changed the term ‘modulate’ to ‘increase’ in the revised manuscript.

**Line 152:** It is unclear what is exactly meant by 'inhibition of hyperpolarization'. It is not clear how reducing spike threshold would inhibit hyperpolarization. Lines 152-156 should be rethought and reworded for clarity.

We have modified the text in the former line 152 to read, “5-HT can also increase discharge rate via mechanisms that reduce the amplitude of slow and medium afterhyperpolarisation phases that follow the action potential (Hounsgaard et al., 1988; Hounsgaard & Kiehn, 1989; Bayliss et al., 1995; Wikstrom et al., 1995; Grunnet et al., 2004)”.

Similarly, we have reworded the sentences that follow this statement to improve clarity in our discussion, “Although 5-HT can reduce hyperpolarisation by decreasing the threshold for generating a Na+ based action potentials (Fedirchuk & Dai, 2004), 5-HT also generates a Ca+ dependent plateau potential by reducing a K+ current responsible for slow afterhyperpolarisation (Hounsgaard & Kiehn, 1989).

**Line 160:** the DLF is not a specific analogue of the raphe-spinal pathway, but does contain descending serotonergic fibres, in addition to many other. This might be misleading as written.
We have changed our description of the dorsolateral funiculus to, “DLF, contains descending serotonergic fibres”.

**Line 167: which neuron?**

We have changed the term ‘neuron’ to ‘motoneurone’ in the revised manuscript.

**Line 169:** An additional inhibitory and activity dependent mechanism mediated by dynamic sodium potassium ATPase pumps has also been demonstrated by the Sillar lab for control of motoneuron activity in Xenopus tadpoles (Zhang et al. 2012; 2015). Importantly, this inhibitory pump is under the control of 5HT receptors (Hachoumi et al., 2022). Similar inhibitory pump dynamics have also been demonstrated in mammalian motoneurons and can be modulated by dopamine (Picton et al., 2016). These studies are worth highlighting for intrinsic cellular mechanisms that could contribute to the inhibitory (and activity dependent) regulation of motoneuron excitability.

We have included the following sentences to the end of this paragraph:

“Finally, other activity-dependent mechanisms may be influenced by serotonin. Sodium-potassium pump activity can cause inhibitory effects on motoneurone discharge during rhythmic activity (Zhang & Sillar, 2012; Zhang et al., 2015; Picton and Sillar, 2016). In tadpoles, this effect can be modulated bidirectionally through 5-HT2A and 5-HT7 receptors (Hachoumi et al., 2022) although this has not yet been demonstrated in mammals”.

**Line 172:** by 'increased hyperpolarisation' do you mean that the resting membrane potential was hyperpolarized? If so, this should be reworded for clarity.

The reviewer is correct. We have amended our terminology to indicate that 5-HT injected in the vicinity of the motoneurone caused hyperpolarisation of the resting membrane.

**Line 174:** while the work was conducted in JF Perrier's lab, I think it is important to acknowledge and the credit should be drawn to the first author, F Cotel, who likely performed the majority of the work. Please rephrase.

We have changed this sentence to read, “An underlying mechanism of 5-HT-mediated inhibition of motoneurone activity was clarified in a series of elegant experiments by Cotel and Perrier”.

**Line 189:** An interesting observation that I think is worth mention, that 5HT fibres have been reported at the AIS of rodent motoneurons (Deardorff, Romer, and Fyffe, 2021), which is in contrast to that has been suggested by Cotel et al. It is possible that this is a species difference. Alternatively, it might be due to differences in motoneuron types, which have not really been explored. Regardless, it still provides a means for direct compartmental modulation of channels that are expressed at the AIS, which differ from those expressed in the dendrites.
We agree with the referee that this is an important consideration. We have amended our text to read, “However, it is worth noting that these findings are only reported for adult turtle motoneurones and may not be applicable for other species. Indeed, a recent study have identified 5-HT boutons present on the AIS of rodent motoneurones (Deardorff et al., 2021), and compartmentalisation of 5-HT receptor subtypes on human motoneurones is yet to be detailed”.

Line 224: Indeed, previous work has demonstrated contributions of CaV and NaV channels to PIC and PIC-mediated firing properties, and historically it was thought that PICs were relatively straight forward. However more recent work from the Brocard group has suggested that it might be a bit more complex than initially thought as other ion channels can contribute to PIC and PIC-mediated intrinsic properties. Examples include activation of TRPM5 (Bos et al., 2021) and inactivation of Kv1.2 (Bos et al., 2018). Further, M-type potassium currents (mediated by KCNQ channels) influence measures of PIC and their effect on intrinsic properties and oppose NaV1.6-mediated inward currents in excitatory interneurons. While this direct interaction has not been shown in motoneurons, it is worth noting given that motoneurons do express KCNQ channels in somatodendritic and AIS compartments alongside NaV1.6 channels (Verneuril et al., 2020). Whilst historically it was thought that PICs were straightforward, it is becoming more apparent that this is not the case as multiple channels contribute to and even oppose their actions in parallel. A similar argument could be made for modulation of 'PIC-channels' as changes in PIC-mediated intrinsic properties could be mediated through changes in some of these other channels. I highly recommend checking these works out and think that these points are worth highlighting in this review.

We have included the sentence below to highlight evidence of more complex mechanisms of PIC-like non-linearities in motoneurone behaviour.

“However, recent evidence shows that non-linearities in motoneurone discharge may have more complex mechanisms including activation of TRPM5 and inactivation of Kv1.2 channels (Bos et al., 2018, Bos et al., 2021)”.

Line 249: I would suggest softening this statement from PICs mediate serotonin's effect on motor performance, to PICs may partially contribute to serotonin's effect on motor performance. As previously highlighted, many other ion channels can be influenced by serotonin.

We agree that this statement was too strong, so we now suggest that “PICs may be partially responsible for 5-HT mediated increases in voluntary activation”.

Line 251: PICs and their influence on motoneuron intrinsic properties differ between motoneuron subtypes. This might be a good spot to highlight some of these works (Lee and Heckman 1998, Huh et al. 2017; Sharples and Miles 2021). I think it is also worth highlighting that motoneuron subtypes express different complements of ion channels and possibly (although not known) express different 5HT receptors or have inputs to different regions. Along these lines, studies of different muscles with varying muscle fibre
compositions (eg. Soleus, TA, etc) may also respond differently to neuromodulation. These could possibly contribute to task-differences in function and neuromodulatory control.

These comments have provided a valuable inclusion for our manuscript. We have amended our text to the following:

“These findings provide support that PIC activation in humans is linked to voluntary drive and hence 5-HT release. The difference between gastrocnemius and soleus also highlights that PICs, and their influence on motoneuron intrinsic properties, differ between muscles and motoneuron subtypes (Lee & Heckman, 1998; Huh et al., 2017).”

**Line 253-256: This statement isn't very clear. I suggest breaking it up a bit.**

We have rewritten this statement to improve the clarity of the text:

“However, it is known that PICs associated with gastrocnemius medialis increase from 10% to 20% MVC, and PICs associated with soleus increase from 10% to 30% MVC, during slow ramped plantarflexions (Orssatto et al., 2021b). These findings provide support that PIC activation in humans is linked to voluntary drive and hence 5-HT release.”

**Line 256: Not to mention that changes in the expression of the PIC channels or those that oppose their actions could also contribute.**

We are not sure what the reviewer is suggesting here. Acute changes in Delta F and maximal voluntary force are seen after 5 muscle stretches of ~1 min each and then recover over ~10 min. We have not amended the manuscript based on this comment.

**Line 313: suggest toning back language from '...in the spinal cord was due to...' to '...in the spinal cord could be due to...' as although plausible, this is purely speculative.**

We have changed the phrase ‘in the spinal cord was due to’ to ‘in the spinal cord could be due to’ in the revised manuscript.

**Line 348: Modulated 'by' 5HT.**

We have changed the phrase ‘modulated with 5-HT’ to ‘modulated by 5-HT’.

**Line 369-373: Maybe not such a recent idea with the citations provided approaching 15 years ago. Suggest rephrasing.**

We have changed the phrase ‘A recent proposal’ to ‘A reasonable proposal’.
This paragraph ends kind of open. Is there a possibility to capitalise on new genetic tools in rodents that allow for identification and manipulation of defined interneuron subtypes to advance these ideas further? Are there new methods/technologies/techniques in humans that might provide some insight?

We agree that this paragraph finished quite open-endedly. However, we believe that the simple explanation for the open-ended paragraph was its lack of conclusion. In particular, the final sentence did not emphasis the take home message from the paragraph. We have changed the final sentence in this paragraph to read, “Thus, it is possible that a 5-HT mechanism involving inhibitory spinal circuitry can regulate the amplitude of agonist and antagonist muscle contractions”.

I'm not entirely sure that this question was answered in this conclusion paragraph. Indeed, challenges are highlighted, but I think it would be worthwhile to outright statement that might provide more of an answer to this question.

We have changed this paragraph to provide more direction for the reader:

“Animal experiments provide clear evidence that 5-HT is a potent modulator of spinal circuits and motoneurone output. However, the effects of 5-HT on voluntary muscle activity in humans are less clear. Effects on motor performance during whole-body exercise are inconsistent. This is not to say that serotonergic neuromodulation does not matter for humans, but instead highlights the challenges associated with studying how a complex neuromodulatory system acts during muscle contractions. Controlled experiments using single-joint, single muscle, contraction protocols have found that maximal force can be changed by altering 5-HT activity in the CNS. The differences observed in voluntary activation are small but are present despite an otherwise intact system and the actions of other neuromodulators, including noradrenaline. This suggests that serotonin has a non-redundant role in maximal voluntary contractions but still begs the question of exactly how important it is in typical motor tasks. Nonetheless, 5-HT-related changes in muscle activation typically emerge with strong contractions for both the unfatigued and fatigued motor system. Thus, it appears that the magnitude of descending drive to the muscle may be aligned with the level of 5-HT neuromodulation in humans. Indeed, we are beginning to reveal evidence where 5-HT-effects may be scaled to the intensity of muscle activation in humans (Goodlich et al., 2022; Henderson et al., 2022)”.

Line 378: neuromodulation of what? This sentence is rather vague as 5HT is a neuromodulator and it can be inferred that it would contribute to neuromodulation. I would suggest stating that 5HT is a potent modulator of spinal circuits and motoneurone output.

As suggested, we have changed this sentence to, “Animal experiments provide clear evidence that 5-HT is a potent modulator of spinal circuits and motoneurone output”.

Line 393-396: this sentence is a little misleading as it implies that pharmacological manipulation of neurotransmitters in the CNS is unique to humans. However, I think that the main point is detailed in the sentence that follows. Perhaps consider rephrasing these two sentences.
This was a poorly worded sentence where we should not have used the word ‘unique’. We have removed this term and stated in the revised manuscript:

Participant safety is an additional challenge in human experiments that use pharmacology to manipulate neurotransmitter activity. Human studies must operate within a window of safe drug administration, and typically use therapeutic doses of 5-HT modulating medications. Thus, very little is known about how dosage effects influence 5-HT activity in humans.

Figure 1: I think this figure is a little problematic as it attempts to synthesize data from multiple species (turtle and mammals). It should be made clearer in the figure itself (in addition to caption) where these data are derived otherwise it is a bit misleading. As highlighted above, it should be highlighted that the DLF contains descending serotonergic fibres and is not simply an analogue of the raphe spinal pathway.

We thank Referee #3 for this suggestion. Please note that the intent of the figure was to provide an overview of the 5-HT system and not to provide specific details across species. If we were to modify the figure itself to highlight where all of the data was derived from, it would compromise the layout and clarity of its content. Instead, we have highlighted for caption 1A that the subsequent information is derived from the mammalian spinal cord, and for caption 1B that the subsequent information is derived from preparations of the adult turtle spinal cord. The source reference for the turtle study is listed at the bottom of the caption.

REFEREE #2

It was with pleasure that I read the work submitted by Kavanagh and Taylor entitled "Voluntary activation of muscle in humans: does serotonergic neuromodulation matter?" For many years, the field has been left wondering if PICs that are facilitated by 5HT are actually relevant to human function. This topical review starts to address this, and other, aspects of 5HT neuromodulation during normal and fatigued motor output. It is with great certainty that I can say that this article will be highly read and cited. Great work! Below, I have provided some specific comments (with page ; and line [l]) in an attempt to improve the quality of the manuscript.

We thank Referee #2 for these kind comments. We believe that our Topical Review has significantly improved with the recommendations that the referee has made.

p2; l57: Ionotropic/neuromodulatory is not necessarily a class of input, rather they are inputs that activate classes of receptors. The inputs activate either ionotropic or metabotropic receptors and neuromodulatory inputs would predominantly activate those of the metabotropic class. Slight rewording for clarity would be helpful here.

We have modified this sentence to read, “Synaptic inputs to motoneurones will typically activate two classes of receptors: ionotopic and neuromodulatory”.

p5; l137: is this supposed to refer to rates of discharge?
The referee is correct. We have modified the sentence to clarify that we are referring to discharge rate, “Incremental increases in treadmill walking speed correspond to incremental increases in the discharge rate of single raphe-spinal fibres”.

Section ending on l142: The discussion of the role of 5ht on the dorsal cord seems a little light - one might consider a slight expansion of this topic here so that the (predominantly) inhibitory effects of 5ht on sensory transmission can be appreciated.

We have added additional information to this paragraph. In doing so, we have been cautious with the amount of information that we present. In particular, the role that 5-HT plays in regulating afferent and interneuron excitability is extraordinarily complex, and to do justice to the topic would require a review by itself. We have included the following information in the revised manuscript:

“5-HT release into the dorsal horn and intermediate zone of the spinal cord can cause remarkably complex outcomes at the neuronal level, as 5-HT can either depress or facilitate transmission in afferent fibres (Belcher et al., 1978; Jordan et al., 1979; Todd & Millar, 1983), and the response to 5-HT can differ depending on the threshold for afferent activation (Belcher et al., 1978; Jankowska et al., 1993). Adding further complexity to our understanding of how 5-HT regulates sensory neurone in the spinal cord is that 5-HT effects may be exerted pre-synaptically and post-synaptically. In particular, actions of muscle spindle (Ia fibres) and tendon organ (II fibres) afferents on spinal interneurons by 5-HT is dependent on the type of the afferent that is activated and the functional type of the interneuron it is connected to (Jankowska et al., 2000). Thus, it is difficult to predict how motor function may be influenced by the actions of 5-HT on afferent neurons and interneurons”.

p7; l207-209: It may be nice to put this magnitude of change into perspective for the naive reader. If a healthy young adult has near complete activation of the biceps (near 100%; see pre-fatigue %VA in Fig 3) then we would not expect that they could gain more than a percent or two as they cannot have >complete activation (theoretical, I know). Other muscles, with lower %VA, may show greater change.

We agree that more detail could have been provided to give our voluntary activation data context for a naïve audience. Hence, we have modified the text as follows:

A finding of only small drug-related increases in voluntary activation is perhaps not surprising, as increases in motoneurone excitation during near-maximal contraction intensities produce only small changes in interpolated twitch amplitude (Herbert & Gandevia, 1999). Hence, voluntary activation of the biceps (that is calculated from interpolated twitches) may be as high as 98% or 99% in healthy individuals, and any intervention that may increase activation is limited by a very close ceiling (i.e. 100% voluntary activation).

p8; l235: put "i.e. the motoneurone's hysteresis" in parenthesis?

We have placed these words in parenthesis in the revised manuscript.
Although seldomly considered in the literature, the threshold of the PIC can vary and may be a key contributor to MN recruitment. Please refer to the discussion of Afsharipour et al 2021

This is an excellent suggestion and we have modified the text to read, “PICs provide little drive to the motoneurone at recruitment as initiation of an action potential and the activation of PICs occur at a similar membrane potential (but see Afsharpour et al 2020 for important nuances with regard to variations in PIC threshold).”

Section ending l375: I suggest updating this section with reference to the the emerging findings from both CJ Heckman's lab and from the second author's lab (references below). These findings, albeit preliminary in nature, both support the notion that afferent feedback may dampen the effects of PICs in humans: Pearcey et al. 2020 - Exploring the effects of Ia reciprocal inhibition on neuromodulatory commands in the human lower limb (https://doi.org/10.1096/fasebj.2020.34.s1.09445); Mesquita et al. 2022 - Effects of reciprocal inhibition and whole-body relaxation on persistent inward current estimated by two different methods (https://doi.org/10.1113/JP282765)

We have included the following text in this section that highlights the findings of Pearcey et al and Mesquita et al.:

“The notion that reciprocal inhibition can dampen the effects of PICs has also been demonstrated in humans, where 128 Hz vibration of the tibialis anterior tendon to activate dorsiflexor muscle spindle afferents decreases ΔF for both the soleus and medial gastrocnemius during 30% MVC plantarflexions, and vibration of the Achilles tendon decreases ΔF for the tibialis anterior during 30% MVC dorsiflexions (Pearcey et al., 2020). Another human study found that 1 Hz electrical stimulation of the common peroneal nerve also has the capacity to reduce ΔF in medial gastrocnemius motor units in healthy individuals, which builds further support that Ia reciprocal inhibition reduces the contribution of PICs to MU firing in humans. (Mesquita et al., 2022)”.

REFEREE #3

This review provides a comprehensive and balanced overview of the role of 5-HT on muscle activation. Insights from mammalian and from human studies are discussed. The authors do a commendable job of comparing the results from both animal and human studies and identify methodological challenges in testing hypotheses derived from animal work in human studies. The review will be very appreciated by the motor control community. I only have minor comments:

We thank Referee #3 for reviewing our manuscript and providing positive comments. We have carefully considered each comment that has been provided and we have amended the manuscript accordingly.

1. I know that this review looks at voluntary muscle activation but I wonder if there are any insights about 5-HT perhaps modulating reflexive movements in humans and
whether there could be a greater role in that type of movement based on the possibility that serotonergic systems are activated by novel or surprising stimuli.

Given that the focus of this Topical Review is voluntary muscle activation we are cautious to present additional data that would not align with our theme. However, we believe that amendments we have made to the manuscript so far may address Referee #3’s suggestion. In particular, the third paragraph of the section The serotonergic system now reads:

5-HT release into the dorsal horn and intermediate zone of the spinal cord can cause remarkably complex outcomes at the neuronal level, as 5-HT can either depress or facilitate transmission in afferent fibres (Belcher et al., 1978; Jordan et al., 1979; Todd & Millar, 1983), and the response to 5-HT can differ depending on the threshold for afferent activation (Belcher et al., 1978; Jankowska et al., 1993).

Furthermore, our amendment to the section titled Afferent feedback can potentially regulate 5-HT effects at the motoneurone now includes (amongst other things):

The likely candidate for this afferent mechanism is Ia disynaptic reciprocal inhibition evoked by length changes in the antagonist muscle, as other afferents are relatively insensitive to the changes in muscle length that occurred with passive rotation of the cat ankle in the experiment (Hyngstrom et al., 2007). The notion that reciprocal inhibition can dampen the effects of PICs has also been demonstrated in humans, where 128 Hz vibration of the tibialis anterior tendon to activate dorsiflexor muscle spindle afferents decreases ΔF for both the soleus and medial gastrocnemius during 30% MVC plantarflexions, and vibration of the Achilles tendon decreases ΔF for the tibialis anterior during 30% MVC dorsiflexions (Pearcey et al., 2020).

Another human study found that 1 Hz electrical stimulation of the common peroneal nerve also has the capacity to reduce ΔF in medial gastrocnemius motor units in healthy individuals, which builds further support that Ia reciprocal inhibition reduces the contribution of PICs to MU firing in humans. (Mesquita et al., 2022).

2. What about the role of 5-HT in mediating presynaptic inhibition of sensory afferents? Are there insights on this in humans, and does it relate to central fatigue?

Once again, we believe that our amendment to the section The serotonergic system may address this comment. Overall, the role that 5-HT plays on regulating excitability in the sensory system is complex, and the role that 5-HT has in mediating presynaptic inhibition of sensory afferents warrants a review by itself. However, we have briefly described this section:

“Adding further complexity to our understanding of how 5-HT regulates sensory neurone in the spinal cord is the 5-HT effects that may be exerted pre-synaptically and post-synaptically. In particular, actions of muscle spindle (Ia fibres) and tendon organ (II fibres) afferents on spinal interneurons by 5-HT is dependent on the type of the afferent that is activated and the functional type of the interneuron it is connected to (Jankowska et al., 2000). Thus, it is difficult to predict how motor function may be influenced by the actions of 5-HT on afferent neurons and interneurons. Instead, the most direct effects of 5-HT on motor function are via the monosynaptic connections to the motoneurones from the fibres in the raphe-spinal pathway”.
3. If I can make a suggestion to add Bui et al. (2003) (work from Ken Rose lab) to the works cited in line 356.

We do not believe that this reference can be incorporated into the text that the referee has drawn our attention to. Our statement indicates that reciprocal inhibition may be especially critical for regulating PIC amplitude during functional motor activity, where even minor rotations of the ankle joint will reduce PICs by ~50%. The experiments in Bui et al., show that spinal motoneurons, Ia inhibitory interneurons, and Renshaw cells differ in their ability to deliver current from dendritic synapses to the soma and to transmit voltage changes along their dendrites. The work by Bui et al, examines dendritic geometry without providing insight to PICs or reciprocal inhibition.
Dear Dr Kavanagh,

Re: JP-TR-2022-282565R1 “Voluntary activation of muscle in humans: does serotonergic neuromodulation matter?” by Justin J Kavanagh and Janet L Taylor

I am pleased to tell you that your Topical Review article has been accepted for publication in The Journal of Physiology, subject to any modifications to the text that may be required by the Journal Office to conform to House rules.

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Yours sincerely,

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EDITOR COMMENTS
Reviewing Editor:

The authors have done a great job in incorporating the reviewer’s suggestions in their revised manuscript. All reviewers are satisfied and enthusiastic about this review submission.

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REFEREE COMMENTS

Referee #1:

The authors have done an excellent job at addressing my concerns. The manuscript is stronger as a result and I anticipate will be highly cited within the field.

Referee #2:

The authors have done an excellent good job improving their manuscript based on the comments provided from reviewers. Great work!

Referee #3:

The authors have addressed my suggestion appropriately. Apologies for the last suggestion I made. I meant to point the authors towards Bui et al. 2008, not 2003. But it really is a very minor comment, and the revised manuscript is fine.

1st Confidential Review

21-Jun-2022