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PEER-REVIEW REPORT

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Manuscript NO: 60342

Title: Xiangbinfang granules enhance gastric antrum motility via intramuscular interstitial cells of Cajal in mice

Reviewer's code: 00000918
Position: Peer Reviewer
Academic degree: PhD
Professional title: Professor
Reviewer's Country/Territory: Canada
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Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [ ] Anonymous [Y] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

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SPECIFIC COMMENTS TO AUTHORS

This is a very interesting study. I will make comments to increase the value of the paper. 1. Abstract. Please use ICC-MP and not ICC-MY as has always been done by Komuro, Thuneberg, Fauussone Pellegrini, all the big names in ICC research. 2. Abstract methods. Not train, but strain. 3. Resting membrane potential decreased 4. XBF did not depolarize slow waves, it depolarized smooth muscle cells 5. Similarly 6. Why is the ICC-IM the cause? No direct evidence, please discuss more the interactions between vagus and ICC-IM and MMC (Hirst et al., 2002), and see below. 7. The MMC is, as you correctly write, an interdigestive motor pattern. Important references here are Szurszewski (Szurszewski, 1969) and Diamant and Scott (Diamant & Scott, 1987), who recorded the MMC in rats. See also min (Rodriguez-Membrilla et al., 1995). I think that you are the first to record the MMC in the mouse. The true MMC you record in Figure 5A is really beautiful and happens every 15 min or so. You should carefully calculate all parameters of this MMC in all your wild type and WWv animals. I think this is the first really good demonstration of the MMC in wild type and WWv mice. Please compare with the two studies in rats . You will see that inside phase III of the MMC in rats and other animals we see a minute rhythm of activity. Unfortunately, Spencer et al and others have also called this minute rhythm "migrating motor complex", but this is not the real MMC as was originally defined and as you show in figure 5A. So please expand on your description of the MMC as in figure 5A and see what effect XBF has on it. 8. Page 4. Not pacemaker activity and slow waves. Produce slow waves that function as pacemaker activity 9. No one has shown the real MMC in mice except your study. The "migrating motor complex" in mice shown by Spencer et al, shows a rhythmic motor pattern that is not the same as the MMC as described by Szurszewski et al. Please also see (Malysz et al., 1996) (Huizinga et al., 1995). (Huizinga et al., 1998). 10. The MMC in the stomach is

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orchestrated by the vagus (see studies from Nick Diamant), the enteric nervous system and motilin. The ICC-IM are involved in vagal activation of motility (see work from Hirst) so in that way they can influence the MMC. The ICC-IM are also involved in the sensory part vagal activity, see work from Powley. (Chung et al., 1994; Hall et al., 1983; Hall et al., 1986; Powley et al., 2008; Powley et al., 2016) 11. ICC not ICCs, ICC is both single and plural 12. ICC do not have synapses, that is reserved for neurons 13. Page 10, which parameters were significantly different? For example, was the frequency different or were all parameters different? 14. A RMP decreases or increases. The cell depolarizes or hyperpolarizes 15. If atropine abolishes cholinergic neural activity, that should also be abolished by TTX, right? So I do not quite understand why TTX would not mimic the action of atropine 16. Strain gauge experiments: the contraction frequencies are different from the slow wave frequencies, that is likely because not all slow waves cause contraction. But the contractions at 2 or 4 cpm are very likely associated with ICC-MP and since they are sparse in WWV mice, they are lower in amplitude and frequency. 17. Your data on Phase III of the MMC are very interesting and important. Unfortunately you only recorded 30 min, which is a short time for measuring MMC phase III. I hope you sometimes recorded longer and you can tell us about this. You really have to discuss the relationship between ICC-IM and the vagus and the relationship between the vagus and stomach MMC. 18. You also should expand phase III or the MMC and study what kind of contraction patterns there are inside phase III and Phase II just like Diamant and Scott did in the rat. 19. You wrote “The periodic MMC movement turned into a high frequency and high amplitude MMC III phase contraction.” But you did not calculate the specific effects of phase III. Instead you wrote about the fast contractions that are mediated by slow waves. So did the MMC actually disappear and we now see the feeding pattern? 20. The page numbers disappeared. Making it difficult to review and comment 21. You wrote: The results indicated that the I phase contraction of MMC

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occurred in the antrum". I do not understand this. 22. Your wrote: Subsequent treatment with 5 mg of XBF induced the MMC III phase contraction in the gastric antrum of WT mice, which was also observed in W/Wv mice (Fig. 7B, D)," This is very interesting, so you have to better analyse and quantify the effects of the medicine on phase III of the MMC in all conditions, not just calculate the slow wave driven contractions, although that is also important. 23. I am not sure about giving TTX, if you gave enough to kill all nervous activity. Should that not kill the animal? 24. In the discussion you wrote that ICC-MP are present in WWv mice 60%, but in the text you say that they are sparse. Please be more precise and consistent throughout the manuscript. 25. Your wrote: but no regular contraction and typical MMC movement were found in conscious W/Wv mice.". I am not sure, you did find MMC phase III and you did record only for 30 min, so maybe the MMC is there but very low frequency. Do not say that WWv mice do not produce MMCs, because you have measured them!! 26. You write: "In W/Wv mouse intestine, MMC is enhanced by the inhibition of nitric oxide (NO) synthesis [5].", but this is not the same MMC that you record. They call it MMC but it is NOT the real MMC, also the minute rhythm in WWv mice is shown to be blocked by nitric oxide synthesis inhibition. 27. In your study, the typical MMC did NOT disappear in WWv mice, it was just not seen very often, for reasons I do not know for sure, one is the short recording time may be. 28. Your wrote: "of gastric antral through the cholinergic pathway of ICC-IM, rather than the enteric nervous system". Hence it is not rather then, but a combination of ENS and ICC 29. You wrote: "XBF depolarizes SMCs and initiates an action potential, resulting in a rhythmic MMC III phase contraction of gastric antrum in mice." You did not show that XBF induces a regular MMC phase III, please make more and better calculations. 30. Figure 4 F, why did slow wave amplitude not decrease when the depolarization happens? 31. I do not understand the Y axis of Figure 6B 32. This is a very interesting study, and I hope you can make it better because of my comments.

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Reviewer's code: 03476131
Position: Peer Reviewer
Academic degree: PhD
Professional title: Associate Professor
Reviewer's Country/Territory: United States
Author's Country/Territory: China

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SPECIFIC COMMENTS TO AUTHORS

In their manuscript, QiCheng Chen et al. Report how Xiangbinfang granule enhances gastric antrum motility and provides a link of the drug effect via ICC-IM—establishing how XBF mechanism and possible target cell in the gastric antrum would be beneficial in determining the drug effects in GI motility. Although key results should be explained in more detail and proper conclusions should be based on these observations. Major points: 1- The paper can be edited to be more concise, and Grammarly corrected 2- In the results section, the authors describe ICC-IM's absence, but figure 1 (ad and C) clearly shows some ICC-IM. The authors should clearly state these results and avoid using the " disappeared " terminology throughout the MS. "In the gastric antrum of W/Wv, c-Kit immunoreactivity was significantly reduced, the ICC-MY network was sparse, and the ICC-IM disappeared" -The authors should discuss the reduction in ICC-IM that might also produce a functional response in GI tissues, similar to what been observed in the gastric fundus of W/W v mice "Responses to Enteric Motor Neurons in the Gastric Fundus of Mice With Reduced Intramuscular Interstitial Cells of Cajal; Sanders et al. J Neurogastroenterol Motil. 2014" 3- The depolarization effects of XBF observed in WT, and W/Wv mutants are not explained in the MS, and how XBF exerts these effects in w/wv animals with reduced ICC-IM? ….this observation argues against the involvement of ICC-IM in mediating the effects of XBF, or possibly XBF could have other targets? 4- The concentration of Atropine is relatively high? Typically 1 microM is sufficient to inhibit muscarinic receptors effectively. An explanation of why to use 0.5 mM? 5- Figure 4 is confusing, TTX had no effects on XBF mediated enhancement of gastric antrum motility, but atropine completely blocked these effects. This is puzzling; basically, XBF can indirectly release Ach and mediate its effects on ICC, but TTX treatment failed to support this hypothesis. 6- Although

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ICC-IM are important factor in regulating the MMC activity, it seems that the drug works via a neuronal pathway rather than directly affecting ICC. Results are confusing and proper explanations in the MS is needed 7- Figure 5 shows that cyclic MMC-like events in WT animals (describe the propulsion of contents), But the effects of XBF under similar conditions caused increased phasic contractions and not MMC like activity. Can the authors explain the loss of MMC patterns after XBF? Is there a possibility that depolarization by XBF can cause an increase in the contractile status and make it harder to propel content?