Dear Dr. Ma,

We thank you and the reviewers for careful reading of the manuscript and insightful comments. We have revised the manuscript, taking into account all reviewers' comments and suggestions. We have also modified and rearranged other parts of the review for clarity. In particular, we moved the descriptions of biased signaling, compartmentalization of MAPK signaling, and cooperation between G proteins and arrestins into special sections. We hope that you will find the revised version suitable for publication. Point-by-point responses to reviewers' comments are listed below.

Sincerely,

Vsevolod V. Gurevich, PhD
Professor of Pharmacology
Vanderbilt University

Point-by-point responses

Reviewer 1.

The present minireview is a great actual field, and it is expone in clear and concise fashion concepts. Only I has a small numerous posible errors that I remarked d in yellow color on the text.

*Thank you! All indicated errors corrected.*

Reviewer 2.

1) The authors discuss extensively and eloquently the role of G proteins in arrestin-dependent signaling, i.e. how G proteins affect arrestin signaling, but they barely talk about the other way around, i.e. how arrestins affect G protein signaling. In other words, they have largely ignored the role of arrestins in receptor desensitization/dampening of the G protein signal. This aspect of the interplay between the two signal transducers should be elaborated on in one or two paragraphs. In the same vein, the authors hint at the notion that arrestin-mediated MAPK signaling depends on initial G protein activation of the MAP3K in the cascade. If this holds true, wouldn't it then be a biological paradox that arrestins rely on the activity of proteins they normally reduce (G protein activity) for their own signaling?

*Thank you! The section "Arrestin-mediated GPPCR desensitization" describes the first discovered biological role of arrestins: suppression of GPCR coupling to G proteins.*

*Apparent paradox of G proteins and arrestins acting in concert is discussed in the section on their cooperation (on p. 11).*

2) The reviewer agrees with the authors that any given receptor signals through both G proteins and arrestins most likely in parallel (simultaneously) and both signal transducers are needed and utilized to produce a certain cellular effect. This is backed up by several recent investigations, including some referenced by the authors (Refs. #53, #59, #60; also: J Pharmacol Exp Ther. 2015;355:183-190 should be cited) but also quite simply by the fact that no GPCR can be fully activated in the absence of an interacting G protein, as has been demonstrated by the seminal work of Brian Kobilka and of other GPCR structural biologists through the recent years (Annu Rev Biochem. 2018;87:897-919). This strongly suggests that there cannot be any arrestin activation/signaling without prior G protein activation/signaling, which makes the concept of "signaling bias" for GPCRs very difficult to accept, let alone to pursue for therapeutic purposes. The authors allude briefly to the topic of biased signaling on p. 10, middle paragraph, maintaining that design of biased ligands for therapeutic purposes is still attainable, although their logic behind this is not clear, at least not to this reviewer. Please explain in more detail your rationale and view of the concept of biased signaling, especially in light of these recent studies that you also discuss in your present mini-review.

*Thanks! Suggested paper (Littmann et al, 2015) is referenced in the last section (p. 10; ref 74). The paper by Weis et al, 2018, is also discussed on p. 10 (ref 70). We discussed potential signaling bias on pp. 10-11. We added detailed explanations why we believe that biased signaling might still be exploited on pp. 10-11.*

3) The authors should discuss (at least briefly) another aspect with huge physiological significance: does an arrestin-activated MAPK have any different properties (e.g. substrates, cellular effects) from a G protein-activated MAPK? Most of the physiologically relevant studies done so far have not shown any differences in the cellular effects of G protein- vs. arrestin-activated ERKs (e.g. see: Proc Natl Acad Sci U S A. 2009;106:5825-30; J Biol Chem. 2009;284:11953-11962; and other studies). How does this affect the notion of exploiting arrestin-mediated signaling for therapeutic purposes?

*Thanks! This is an important point, but existing evidence is insufficient to make generalizations. We discussed these issues (and mentioned the examples in suggested papers, now refs. 66, 67) on p. 9.*

4) The authors have cited a recent paper on carvedilol-induced Gi coupling to the beta1AR (Ref. #71), as an example of arrestin-mediated signaling dependent on G protein activation. I do not think this study qualifies as a good example, given that a) carvedilol is known to be an inverse agonist for G proteins (Eur Heart J. 1996;17 Suppl B:8-16), and, more importantly, b) this study failed to demonstrate any carvedilol-

bound beta1AR-arrestin interaction or any actual arrestin-dependence of the carvedilol-induced ERK activation.

*We agree that every study, including the study of carvedilol (p. 10, ref 73) has certain caveats. However, we respectfully disagree that that particular study should not be mentioned.*

Minor comments: 1) Please provide the full, correct citations for Refs. #47 & #60.

*Thanks! As the papers came out now, full references are provided.*

2) P. 6, line 3: change "inositol-hexaphosphate" to the correct term "inositol-hexakisphosphate".

*Thanks! Done.*

3) P. 8, par. 2, line 7: correct the typo "arrestin-meditated" to "arrestin-mediated".

*Thanks! Done.*

Reviewer 3

This is an insightful review into the current controversy regarding G protein vs arrestin-mediated signaling by GPCRs. It should be mentioned that in the Grundmann et al. paper that arrestin KO cells have significantly lower ERK1/2 phosphorylation than WT cells (Supplementary Figure 4), consistent with the interpretation of G proteins acting as an initiator of signaling that is propagated by arrestins.

*We thank the reviewer for his/her evaluation of the manuscript. We explicitly mention Suppl Fig. 4 in Grundmann et al paper (p. 8).*

Other comments: Abstract, line 3: HEK292 should be HEK293 GPCR-dependent arrestin signaling section, end of first paragraph: Time dependence of G protein and arrestin signaling.

*Thanks! Done.*

Sometimes G protein-mediated signaling can also have a slow phase, so kinetics alone cannot distinguish G protein- from arrestin-mediated ERK. (Luo, J., Busillo, J. M., and Benovic, J. L. (2008) M3 muscarinic acetylcholine receptor-mediated signaling is regulated by distinct mechanisms. Mol. Pharmacol. 74, 338–347)

*Thanks! We discussed this on p. 5, specifically citing the paper by Lou et al (ref 30).*