“Truth is ever to be found in simplicity, and not in the multiplicity and confusion of things” — Sir Isaac Newton

To the Editor:

We read with interest the recent publication by MORICE *et al.* [1] “The effect of gefapixant, a P2X3 antagonist, on cough reflex sensitivity: a randomised placebo-controlled study” and the accompanying editorial by TURNER and BIRRING [2] “Chronic cough: ATP, afferent pathways and hypersensitivity”. The authors of both publications conclude the results are suggestive of two separate neuronal pathways mediating the cough reflex; however, there are significant issues with this interpretation of the data.

Gefapixant is a first in class therapy that blocks the effects of ATP (adenosine triphosphate) at P2X3 and also P2X2/3 ion channels on sensory nerves, and has already been shown to be highly efficacious at reducing the frequency of coughing in patients with refractory chronic cough [3, 4]. MORICE *et al.* [1] studied the effects of a single dose of gefapixant on experimentally evoked cough in healthy volunteers and patients with refractory chronic cough. Participants underwent four different cough challenges (capsaicin, citric acid, distilled water and ATP) after taking gefapixant and a matched placebo, in a double blinded randomised trial.

This study found gefapixant reduces cough evoked by ATP and distilled water, but not citric acid or capsaicin; distilled water is a hypotonic challenge, which we have shown to be capable of activating airway nerves through release of ATP [5]. The data are therefore consistent with gefapixant blocking P2X3 channels and preventing airway nerve activation by ATP but having no effect on nerve activation by capsaicin or citric acid, which are known to evoke cough *via* a range of other ion channels; capsaicin activates nerves *via* transient receptor vanilloid 1 (TRPV1), whereas citric acid is thought to activate TRPV1, transient receptor potential ankyrin 1 (TRPA1) and acid-sensing ion channels (ASIC) [6, 7]. However, in both the manuscript and the accompanying editorial, the authors misinterpret this data, suggesting these findings indicate two separate peripheral neuronal pathways mediating cough. This is highly unlikely given P2X3 and TRPV1 channels are well known to be expressed by the same airway nerves [8, 9]. Furthermore, the findings are readily explained by the activation of a single nerve pathway expressing both P2X3 and TRPV1 ion channels, with only P2X3 being blocked by gefapixant.

In addition to issues with the data interpretation, there are concerns about the study design. As MORICE *et al.* [10] have previously shown, capsaicin and citric acid cough challenges interact with one another, inducing reduced cough responses to one another, probably *via* tachyphylaxis; these effects persist for at least 6 h; whether similar effects occur with distilled water and ATP is unknown. Therefore, a study designed to include 12 cough challenges on the same day will inevitably be plagued by interactions between all the challenges. This could have been mitigated by randomisation of the order of the challenges to include all the possible combinations and enrolling sufficient patients to represent all possible sequences; unfortunately, this was not done. The authors and commentary writers argue this was not a problem as each subject acted as their own control; however, as not all challenge sequences were represented, a systematic effect of the challenge order influencing the results cannot be ruled out, nor adequately evaluated statistically. We would therefore suggest that this methodological problem in the design should have restricted the interpretation of the data to cautious conclusions, instead of declaring evidence for new peripheral pathways mediating cough.

The peripheral and central mechanisms involved in refractory chronic cough are complex and currently poorly understood. Evidence from pre-clinical models shows that several neuronal pathways are capable of

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Over interpretation of cough challenge data only adds to the confusion in our understanding of the mechanisms underlying chronic cough <http://bit.ly/2X52CQt>

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activating the cough reflex; for example, both chemically sensitive C-fibres and mechanically sensitive A δ fibres play a role. However, they do not support the paradigm of discrete endogenous and exogenous pathways proposed by TURNER and BIRRING [2]. We suggest that the most appropriate interpretation of the data presented by MORICE *et al.* [1] is that, notwithstanding significant shortcomings in the study design, they simply provide clinical evidence that gefapixant blocks the P2X3 but not the TRPV1 receptor in the lung, *i.e.* confirm its known mechanism of action. We would caution that such over-extrapolation of clinical cough challenge data only adds to confusion in the field.

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Author response: The human cough reflex has separate, distinct pathways

From the authors:

We thank J.A. Smith and co-workers for their interest in our paper [1]. Perhaps an even more ancient truth, that of William of Occam [2], should be applied to this discussion.

We were the first to demonstrate that cough challenge testing reveals a fundamental mechanism for clinically important hypertussia, cough reflex hypersensitivity [3]. Whilst we agree the mechanism of this aberrant response remains to be fully elucidated, we believe our study does indeed reveal a fundamental dichotomy within the human cough reflex. That P2X3 and TRPV1 expression occurs within the same neurons appears true in resected vagal nerve fibres; however, we were careful not to claim the anatomical separation that is erroneously suggested by J.A. Smith and co-workers. As we point out “The more central pathways of the vagal afferents through the nodose and jugular ganglia to the solitary nucleus are extremely complex and varied, and exhibit marked plasticity and redundancy in disease. In this environment, the interpretation of cough challenge studies must be undertaken with care.” To use Occam’s razor, the simplest mechanism to explain our observations is that there are two biological/pathophysiological cough pathways, one irritant and the other pathological, responsible for the hypertussia seen in chronic cough. Whether these pathways reside in the same or separate neurons remains to be elucidated.

With regard to isolated nerve studies, we are reminded of the old saying that to a physiologist an intact animal is one with all its organs in the same room. In cough, reliance on such models has led to several blind alleyways, as illustrated by the failure in the clinic of studies of TRPV1 [4, 5] and TRPA1 [6] antagonists.

Our study was carefully designed to minimise any effect of repeat challenges with in patient randomisation of challenge order. As we point out, a *post hoc* analysis demonstrated no carry over effect. This objection is doubly surprising since the same findings regarding the effect (or lack of) of gefapixant on capsaicin and ATP challenge has been observed by SMITH *et al.* [7], though we are unable to fully comment on the challenge methodology from this abstract.

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The human cough reflex has separate, distinct pathways <http://bit.ly/2PZgybq>

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