

# **Vanilloid receptor pharmacology in recombinant and primary culture systems**

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# Introduction

- Vanilloid receptors
- Methods in FLIPR™ and FLIPR<sup>384</sup>
- Timecourse of agonist-induced response
- Agonist pharmacology
- Antagonist pharmacology
- Conclusions

# Vanilloid receptors: I

- The vanilloid receptor 1 (VR1) is a ligand-gated ion channel, which plays an important role in nociceptive processing
- The rat vanilloid receptor 1 (rVR1) was recently cloned, stably expressed in HEK293 cells and pharmacologically characterised
- VR1 is also endogenously expressed in rat dorsal root ganglion (DRG) cells

## Vanilloid receptors: II

- Rat DRG primary cultures allow us to determine the physiological relevance of our recombinant system
- Production of rat DRG cultures is labour intensive and offers low yield thus unsuitable to 96 well format
- Using FLIPR<sup>384</sup> affords the opportunity to compare the pharmacology of vanilloid receptors in our recombinant system with that seen in primary cells

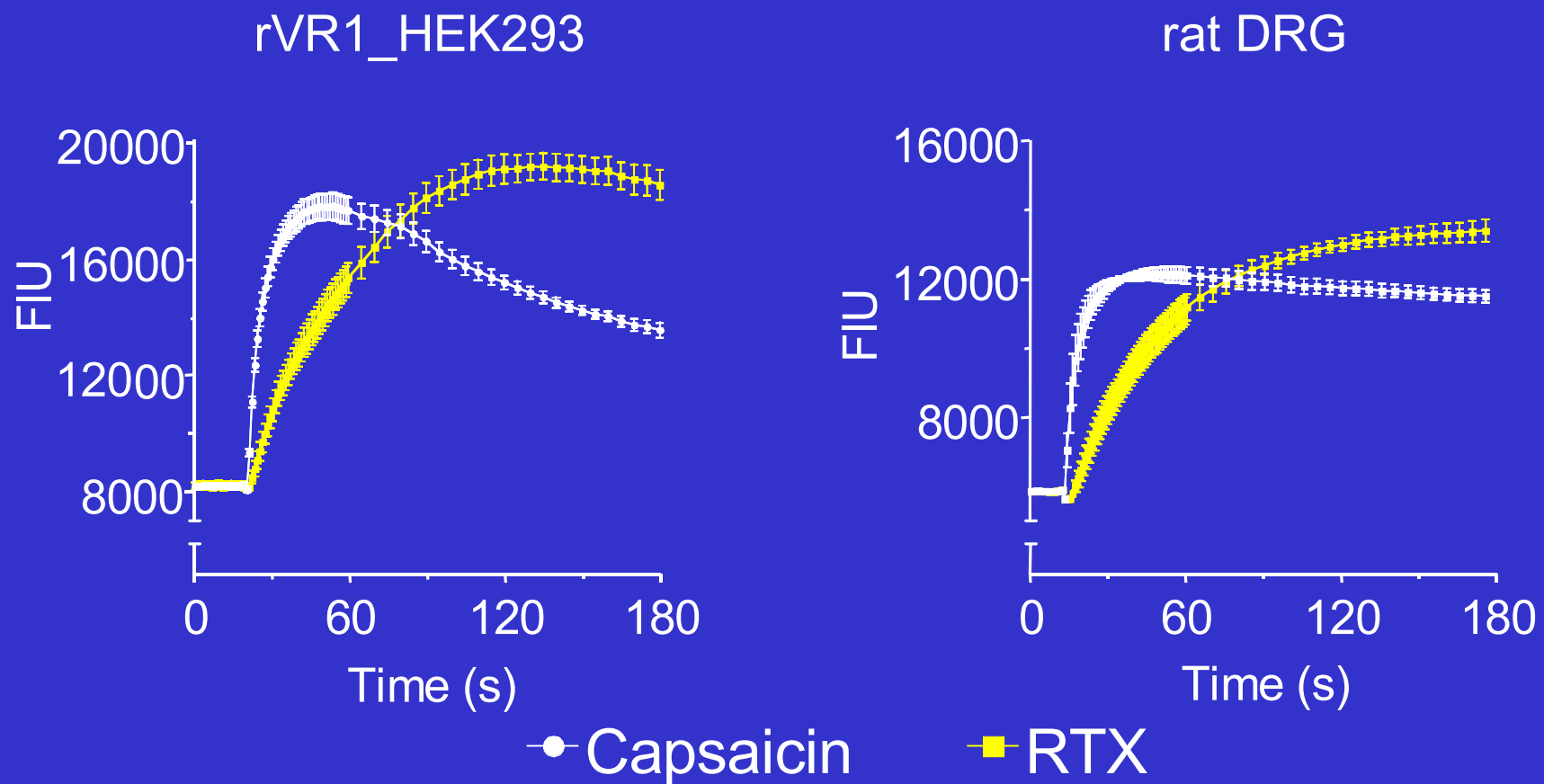
## Method for rat VR1 in FLIPR™

- rVR1\_HEK293 plated out in 96 well black wall, clear bottom plates at 25,000 cells/well overnight
- Loaded with Fluo-3AM [4 $\mu$ M] at 25°C for 120mins
- Washed 4x and resuspended in Tyrodes medium
- Incubated for 30 min at 25°C with either buffer (control) or buffer containing various antagonists
- Cell plates then placed in FLIPR™ for the addition of various agonists

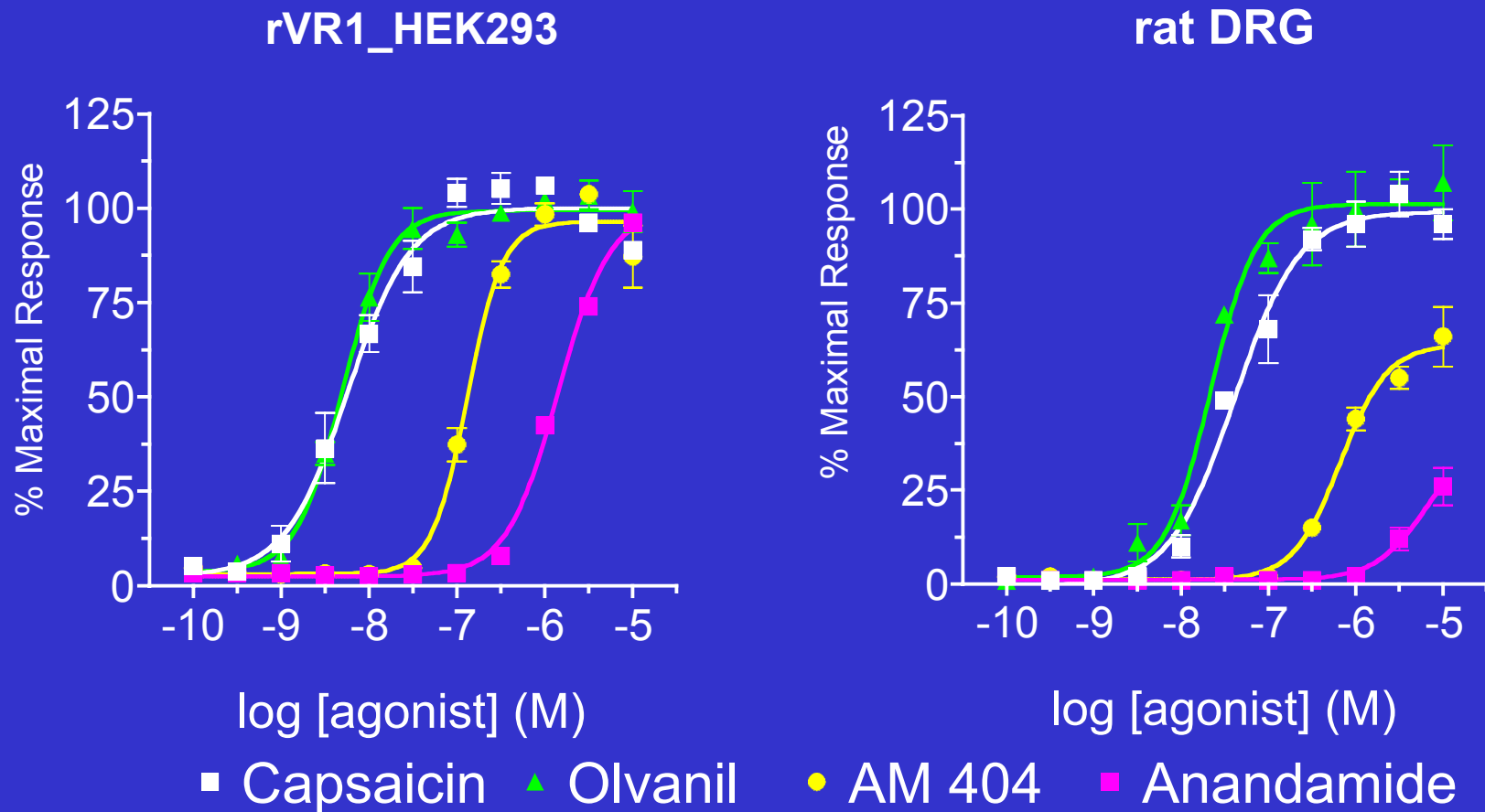
## Methods for rat DRG in FLIPR<sup>384</sup>

- Rat DRG cells harvested, dissociated and plated out at 5,000 cells/well in laminin coated 384 well black wall, clear bottom plates overnight
- Loaded with Fluo-4AM [4 $\mu$ M] at 37°C for 1hr
- Washed 4x and resuspended in Tyrodes medium
- Incubated for 30 min at 25°C with either buffer (control) or buffer containing various antagonists
- Cell plates then placed in FLIPR<sup>384</sup> for the addition of various agonists

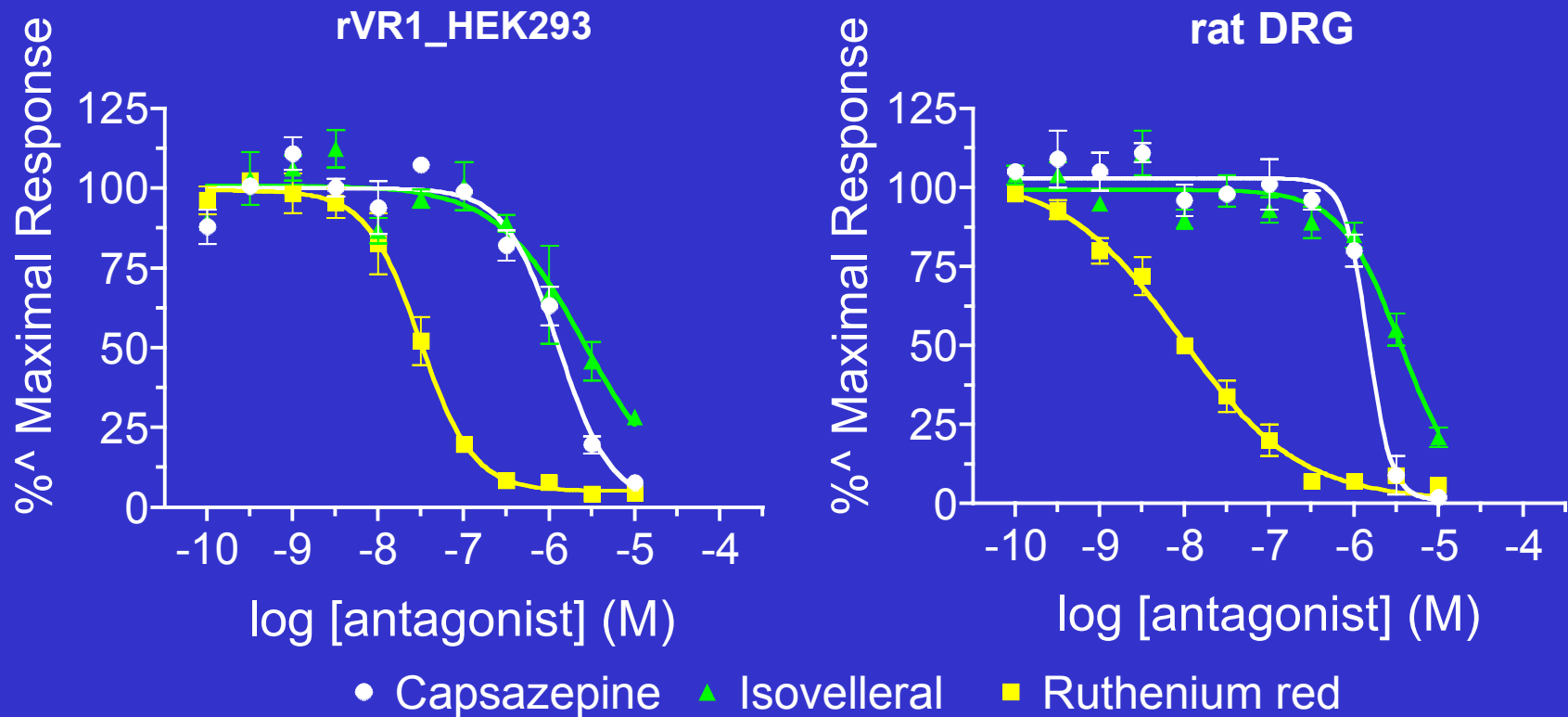
# Agonist Response Temporal Profile



# Agonist Concentration Effect Curves in rVR1\_HEK293 and rat DRG cells



# Antagonist Concentration Effect Curves in rVR1\_HEK293 and rat DRG cells



# Agonist Potencies in rVR1\_HEK293 and DRG cells

|            | rVR1_HEK293            |                      | rat DRG                |                      |
|------------|------------------------|----------------------|------------------------|----------------------|
|            | pEC <sub>50</sub>      | E <sub>max</sub>     | pEC <sub>50</sub>      | E <sub>max</sub>     |
| Capsaicin  | 7.68 <sub>±</sub> 0.11 | 1.0 <sub>±</sub> 0.1 | 7.45 <sub>±</sub> 0.10 | 1.0 <sub>±</sub> 0.1 |
| Olvaniil   | 7.69 <sub>±</sub> 0.11 | 1.0 <sub>±</sub> 0.1 | 7.55 <sub>±</sub> 0.07 | 1.1 <sub>±</sub> 0.1 |
| RTX        | 8.73 <sub>±</sub> 0.15 | 1.2 <sub>±</sub> 0.1 | 7.96 <sub>±</sub> 0.02 | 1.2 <sub>±</sub> 0.1 |
| AM 404     | 6.54 <sub>±</sub> 0.12 | 1.0 <sub>±</sub> 0.1 | 6.10 <sub>±</sub> 0.13 | 0.6 <sub>±</sub> 0.1 |
| Anandamide | 5.73 <sub>±</sub> 0.04 | 1.0 <sub>±</sub> 0.1 | 5.42 <sub>±</sub> 0.08 | 0.3 <sub>±</sub> 0.1 |

Data are mean<sub>±</sub>s.e.mean, n=4-6

## Antagonist Affinities ( $pK_B$ ) in rVR1\_HEK293 and DRG cells

|               | rVR1_HEK293     | rat DRG         |
|---------------|-----------------|-----------------|
| Capsazepine   | 6.91 $\pm$ 0.08 | 6.42 $\pm$ 0.02 |
| Ruthenium red | 8.54 $\pm$ 0.08 | 8.60 $\pm$ 0.06 |
| Isovelleral   | 6.53 $\pm$ 0.07 | 6.08 $\pm$ 0.03 |

Data are mean $\pm$ s.e.mean, n=4-6

## Conclusion: I

- We have cloned the rat vanilloid receptor, which is important in nociceptive processing
- We have pharmacologically characterised the rat VR1 receptor in both a stably transfected cell line and an endogenous primary culture system
- The responses in both systems are comparable both in terms of the timecourse of the agonist-induced response and agonist rank order of potency

## Conclusion: II

- Levels of VR1 receptor expression may alter agonist relative efficacies
- We have shown that antagonist pharmacology defined by our recombinant system mirrors that seen in the more physiologically relevant DRG system
- We have developed a robust, reproducible assay in FLIPR<sup>384</sup> which allows the characterisation of the rat VR1 receptor in a physiologically relevant primary cell system

# Acknowledgements

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rat DRG cells

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