Supporting Information

An *in vitro* and *in vivo* investigation of bivalent ligands that display preferential binding and functional activity for different melanocortin receptor homodimers

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Supporting Information. AlphaScreen® data normalization (S1-S2), Latin-Square (crossover) feeding paradigm (S3), Male and Female Mice ICV Feeding (0-72 hours) (S4). Graphical representation of partial functional receptor activation (S5).

ALPHAScreen Assay Data Normalization

The AlphaScreen® assay is a competition assay between biotinylated cAMP which is part of the assay kit and intracellular cAMP which is produced by the cells in response to agonist ligands. Because the AlphaScreen® assay is a competition assay, it results in decreasing signal with increasing cAMP functional response due to receptor activation. This is inconsistent with standard functional assays which result in increasing signal with increasing receptor activation. We, therefore, normalized the data to flip data curves to be consistent with the literature. Of note, the EC_{50} values reported were obtained prior to data normalization. Data normalization was only used for illustration purposes and did not have a significant effect on the EC_{50} values.

Data was normalized by taking the raw value of each well, subtracting the averaged maximal response of NDP-MSH (normally responses at 10^{-6} and 10^{-7}). This value was then divided by the maximal response subtracting the average minimal response (Basal values and/or values of NDP-

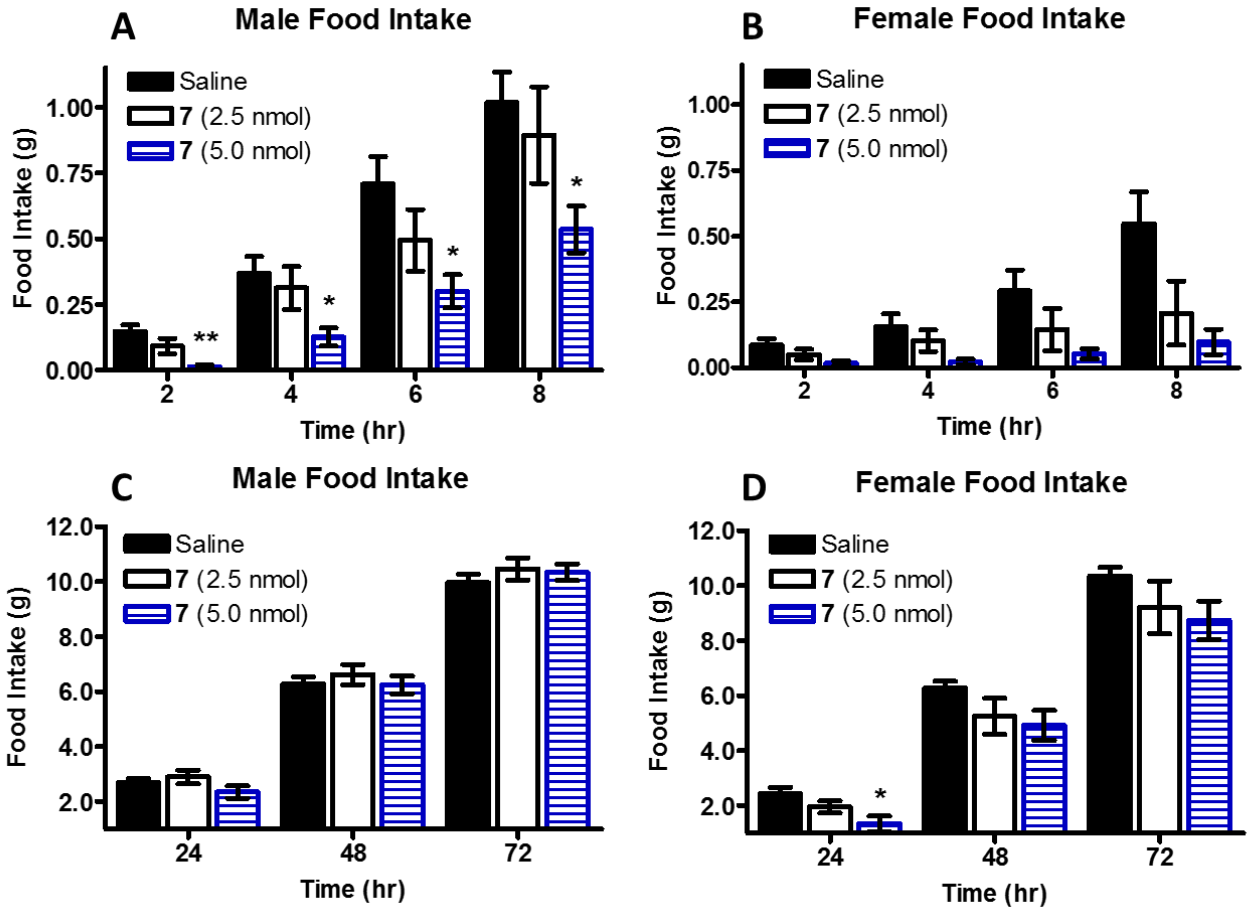
MSH at 10^{-12}). This value was then subtracted from 1 to flip the response and multiplied by 100 to result in % response of NDP-MSH. The equation is as follows:

% Response of NDP-MSH = 1-[[(Raw Value)-(Average Max Response)]/[(Average Minimal Response)-(Average Max Reponses)]] X 100

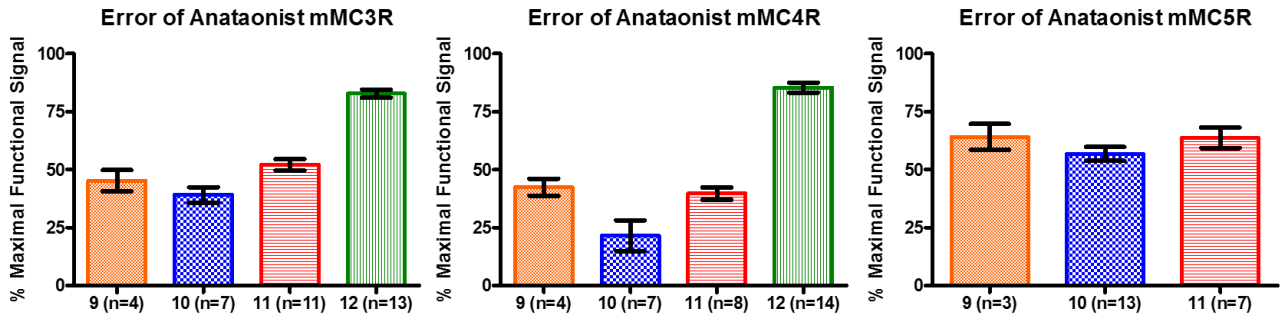
Latin-square (Cross-over) Paradigm

	Group 1	Group 2
Day 1	Saline	**7** (5 nmol)
Day 2	**7** (5 nmol)	Saline
Day 3	Saline	**7** (2.5 nmol)
Day 4	**7** (2.5 nmol)	Saline

All animals received both compound and saline in this order. The same animals were used throughout the experiment. There was at a least 6 day washout period between injections.



Supp. Info. Figure 1. Cumulative food intake following intracerebroventricular administration of either saline (n=16 male; 8 female) or **7** in saline (n=8 male; 4 female) in wild type mice. (**A)** Male dose response of food intake in first 8 hours as appear in primary manuscript (appears here for comparison.) **(B)** Female dose response of food intake in first 8 hours. Data is not significant. (**C**) Male dose response of food intake between 24-72 hours. Data is not significant. (**D**) Female dose response of food intake between 24-72 hours. The 24 hr time point is significant ($p<0.05$). Data is shown as mean ± SEM. Data was analyzed using the PRISM program (v4.0; GraphPad Inc.) by a one-way ANOVA folled by a Bonferroni post test in order to compare individual doses to saline administration. *$p<0.05$, ** $p=0.01$.



Supp. Info. Figure 2. Partial functional receptor activation seen in **Figure 6** and **Table 3** reported as the Mean±SEM of at least three independent experiments. Percentage (%) is the amount of activity relative to maximal NDP-MSH response was observed at 100 μM.