SUPPLEMENTARY MATERIAL

Materials and methods

Confirmation of ICV cannula placements

For the ICV experiments, the correct placement of the guide cannulae in the rats was confirmed by injecting rats with 1 µl of Angiotensin II (100 ng/µl) via the ICV route and monitored for a drinking response for 10 min post-injection. Rats with a positive drinking response (onset of drinking ≤ 1 minute; ≥ 5 ml of water in 10-min time period) were used for the study (Gosnell *et al*, 1990; Choi *et al*, 2003).

Histology

Upon completion of the experiments the animals were euthanized by CO2 inhalation; then, their brains were quickly removed and snap-frozen at -20 °C in isopentane. The brains were then stored at -80°C for further histological examination. Serial 20 µm brain coronal sections were cut on a cryostat (CM 1950, Leica Biosystems, Germany) and mounted on Color Frosted microscope slides. The positions of the microinjection sites were evaluated according to the atlas of Paxinos and Watson (1998) and only behavioural data obtained from rats which had the cannulae correctly placed in both hemispheres of the brain were analysed (N=7 for DH; N=9 for VH and N=22 for MeA).

Figure legends

Figure S1: Effect of intracerebroventricular injection of L822429 (0.0, 7.5, 15.0 μg/rat) on alcohol self-administration in msP rats under FR-1 schedule of reinforcement. Data are the mean ± SEM of total number of rewards earned in 30 min (N=8). L822429 did not reduce the number of rewards at these doses. No significant differences were found between the treatment groups. Vehicle-treated group: white bar; drug-treated groups: black bars.

Figure S2: Microinjections into the Medial Amygdala were performed within the highlighted area. Taking bregma as reference point, the following coordinates were used in accordance with the atlas of Paxinos and Watson, 1998: Antero-Posterior= 2.7 mm; Medio-Lateral= 3.4 mm; Dorso-Ventral= 9.0 mm.

Figure S1

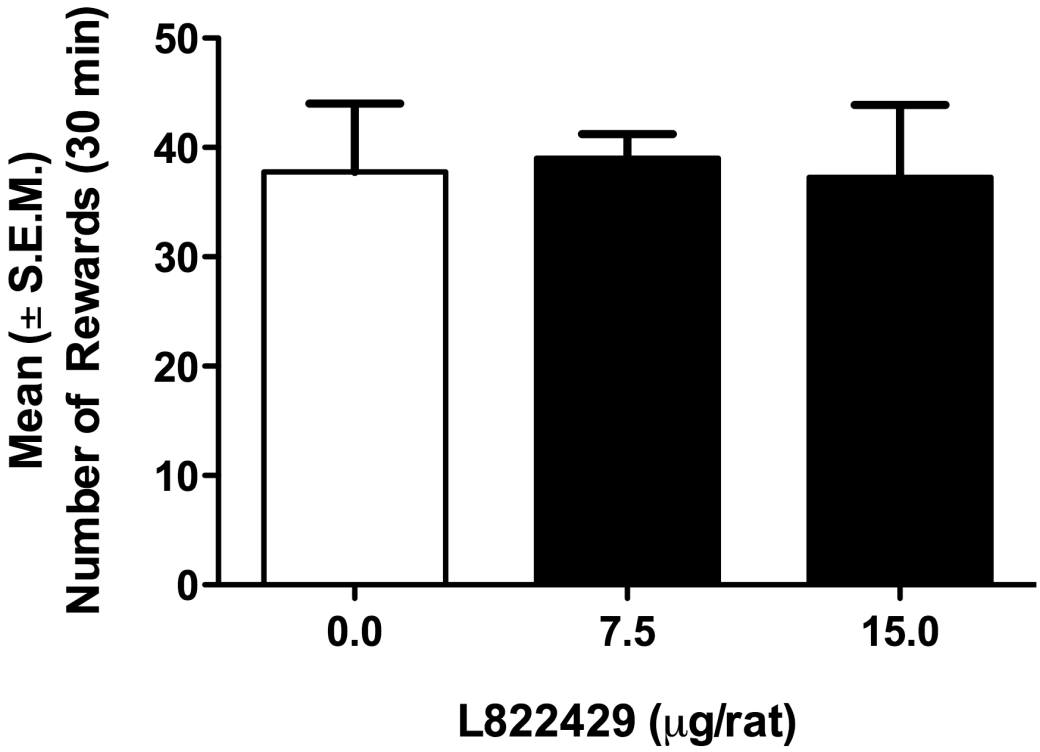
Mean (± S.E.M.)
Number of Rewards (30 min)
50
40
30
20
10
0
0.0
7.5
15.0
L822429 (μg/rat)

Figure S2