Effect on reward processing of microinjections of gastrin-releasing peptide and amphetamine into the intra-nucleus accumbens

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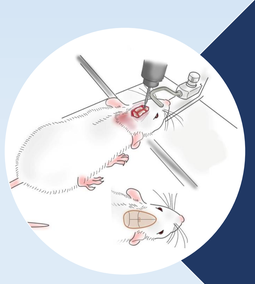
Introduction

Gastrin-releasing peptide (GRP) is the mammalian counter of the amphibian peptide, bombesin (BB). Receptors for GRP, named BB2, are widespread throughout the central nervous system with particular abundance in the nucleus accumbens (NAcc). In addition, a portion of BB2 receptors at the NAcc are located on dopamine (DA) terminals. A recent study in our lab revealed that an injection of GRP (1.7μg/μL) directed at the NAcc caused an immediate and significant increase in DA release at this site compared to saline injection as measured using *in vivo* microdialysis. This finding provided compelling evidence of involvement of the BB family of peptides in reward processes. Thus, the objective of this experiment is to extend these findings by assessing the effect of intra-NAcc microinjections of GRP and amphetamine in a behavioural paradigm linked to reward and motivation, namely the progressive ratio (PR). The PR paradigm utilizes rat operant chambers that are placed inside sound-proof compartments and are equipped with two retractable levers, a sugar pellet dispenser, a house light, and a central ventilation fan. It is hoped that the results will further shed light on the relationship of BB family of peptides and GRP with motivation and reward processing.

Methods

Animals were first reduced to 90% of their *ad libitum weight* and were exposed to sugar pellets in the home cage. The rats were then habituated to standard operant chambers (Med Associates) on a fixed interval (FI) of 30 seconds/pellet. Rats were then shaped to respond on a fixed ratio 1 (FR-1) schedule where each correct response was rewarded with a pellet. After determined to be shaped, rats were increased to a FR-5 schedule. All FR schedules lasted 30 minutes or until 100 rewards. Following FR schedule, rats were trained on a progressive ratio 2 (PR-2) schedule. On PR-2 schedule, the rat was required to increase the number of correct responses by two for each subsequent reward, thus, progressively more effort was needed to earn a reward. The PR-2 sessions ended after an hour or if no reward was obtained in 20 minutes. After seven days on a PR-2 schedule, the rats were returned to *ad libitum* feeding for two days and underwent surgery.

Operant Conditioning

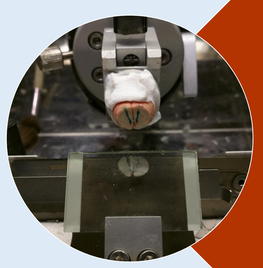


Animals were anesthetised with 2% isoflurane gas and implanted bilaterally with 23 gauge guide cannulae (Plastics One, Roanoke, VA, USA) aimed at the nucleus accumbens (1.70 mm anterior to bregma, 1.60 mm lateral to the midline, and 7.02 mm ventral to the skull, angle 10°). Three stainless steel screws and dental cement were used to anchor the cannulae to the skull. Animals recovered for eight days and were given rectal acetaminophen (50mg/kg) for the first four days, during which they were individually housed, handled and acclimatized to the injection procedure.

Surgery

GRP (Phoenix Pharmaceuticals), and d-amphetamine (Sigma) were dissolved in sterile 0.9% (wt/vol) saline. Amphetamine was used as a positive control as it is a dopamine up-regulator. The drugs (in a volume of 0.5 µl) were infused through 12.5mm injector cannulas into the nucleus accumbens. Infusions were delivered with a microdrive pump (Harvard Apparatus) using polyethylene tubing. The rate of the injection was 1.0 µL/min for a total duration of 60 seconds. An additional 30 seconds were allowed for diffusion.

Microinjections



Following the completion of testing, animals were euthanized with an overdose of isoflurane gas directly followed by decapitation. Brains were collected and frozen on dry ice prior to cryosectioning. Following, the sections were then stained using thionin to verify injection sites and probe placements. Only animals for which all placements were verified were included in the data analysis.

Histology

Results

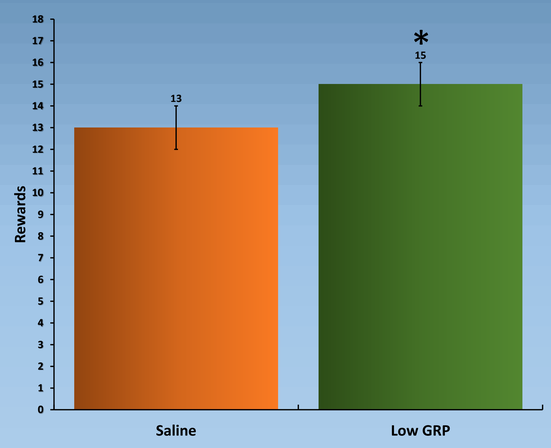


Figure 1: Number of rewards obtained on a PR-2 schedule by rats injected either with a saline solution or 0.435μg/μL of GRP

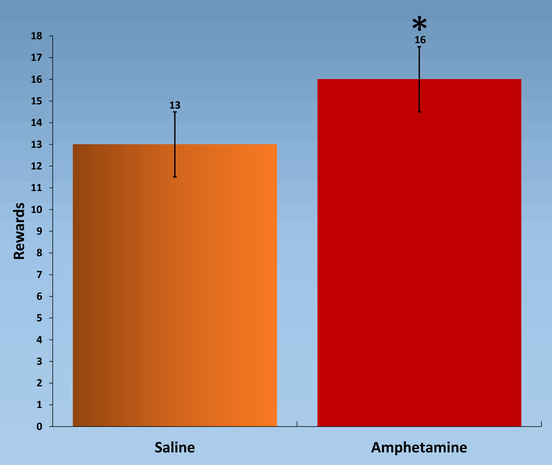


Figure 2: Number of rewards obtained on a PR-2 schedule by rats injected either with a saline solution or 10 μg/μL of amphetamine

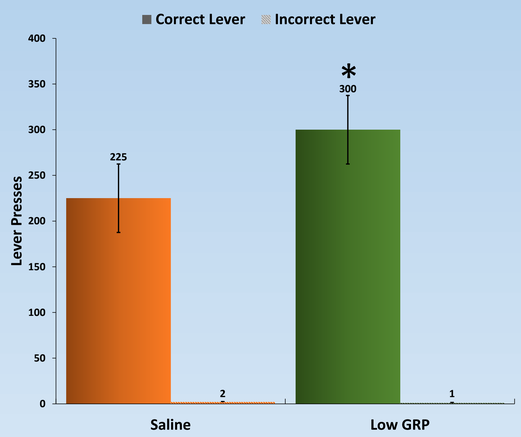


Figure 3: Number of correct and incorrect lever presses on a PR-2 schedule by rats injected either with a saline solution or 0.435μg/μL of GRP

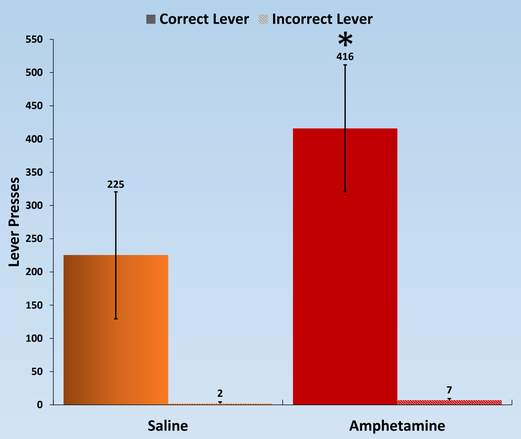


Figure 4: Number of correct and incorrect lever presses on a PR-2 schedule by rats injected either with a saline solution or 10 μg/μL of amphetamine

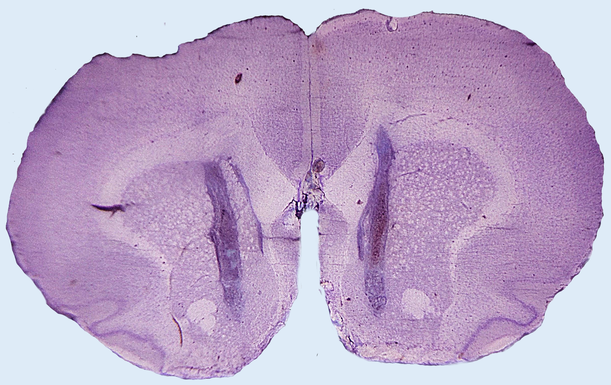


Figure 5: Anterior view of the nucleus accumbens of rat #9 visualizing the injection sites and bilateral cannulae.

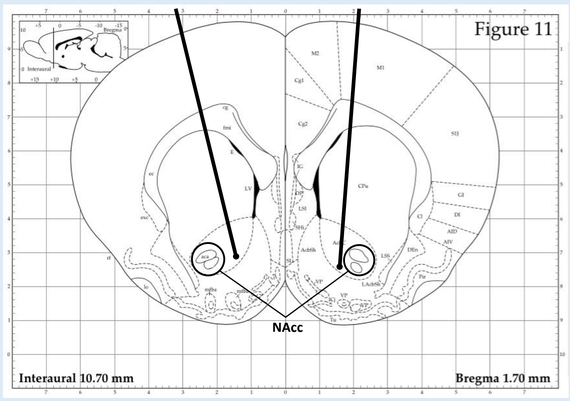


Figure 6: Representation of the bilateral cannulae pathways and injection sites aimed for the intra-nucleus accumbens.

Conclusion

The microinjections of 0.435μg/μL of gastrin-releasing peptide into the intra-nucleus accumbens resulted in an increase in the number of total lever presses, thus, an increase in the rewards obtained on a PR-2 schedule of reinforcement. These findings suggest that GRP increases the motivation of rats to obtain a reward as compared to rats injected with saline solution. Further studies on the effect of BB2 receptor concentration may provide more evidence to this conclusion.

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