SUPPLEMENTARY MATERIALS

Prefrontal cortical GABA modulation of spatial reference and working memory

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Supplementary Methods

Apparatus

The radial maze used in these experiments had a 40 cm diameter octagonal center platform connected to eight equally spaced arms (50 × 9 cm) with a food cup placed at the end of each arm. The maze was placed in a (270 X 330 cm) room with numerous extramaze cues on the walls. On the first 2 days of testing, rats were familiarized to the maze by being placed in the center and allowed to explore for 10 min with no food available, after which they were returned to their home cages and given approximately 20 food reward pellets (Bioserv, Frenchtown, NJ) that were used as reinforcement during training. The arms and center of the maze were wiped down after each rat was removed from the maze.

Behavioral Procedures

For all tasks, an arm entry was recorded when a rat moved down the entire length of an arm and reached the food cup at the end of the arm. The latencies to enter the first arm and to complete the trial were also recorded.

For the Reference/Working memory task, all entries to arms that were never baited were scored as reference memory errors, irrespective of whether a rat had already visited that arm in the current or earlier trials of the session.

For the 8-arm foraging task, over the course of training, rats were discouraged from using a serial selection strategy by distracting them if they chose more than 4 adjacent arms consecutively. By the end of training, none of the rats were using this type of strategy.

Histology

After testing, rats were sacrificed in a CO_2 chamber. Brains were removed and fixed in a 4% formalin solution for at least 24 h. Brains were frozen, sliced in 50 μm sections, mounted, and stained with Cresyl Violet.

Drugs and micoinfusion procedures

All drugs were dissolved in 0.9% saline. One or two days before their first microinfusion test day, rats received a mock infusion procedure, during which obdurators were removed from the guide cannulae, and replaced with stainless steel injectors for 2 min, without an infusion.

Bilateral infusions were made through 30-gauge injectors extending 0.8 mm below the guide cannulae. Saline, bicuculline, or baclofen/muscimol (Sigma-Aldrich, Oakville, Ontario, Canada) were infused at a rate of 0.5 µl/75 s. Following infusions, the injectors were left in place for 1 min to allow for diffusion. Rats were then placed back in their home cages for 10 min, after which, testing commenced. On test days, rats remained on the maze until all baited arms were entered.

Rats subsequently received daily training sessions on their respective task until they again achieved criterion performance. On the day after criterion performance was reestablished, rats received a second counterbalanced infusion of saline, or drug, and this continued until rats had received all designated infusion treatments.

Data Analysis

For the radial maze tasks, the main dependent variable of interest was errors committed during test days. For these analyses, we compared errors made during the first trial of the session to the average number of errors made on trials 2-5, to assess whether treatments disproportionately affected errors made during the latter trials, when rats would be more susceptible to proactive interference from previous trials. These data were analyzed with two-way repeated measures ANOVA, with treatment and trial (1 vs 2-5) as two within-subjects factors. For the Reference/Working memory task, we also analyzed the total number of reference and working memory errors across the 5 trials with a similar ANOVA model, with treatment and error type as two within-subjects factors. For the latency data, we analyzed the time to enter the first arm on each trial (time to initiate) and the average time per subsequent choice with separate two-way ANOVAs, with treatment and trial as within-subjects factors. Lastly, for the spatial discrimination, error data were collated into 5 blocks of 4 trials. These data were analyzed with two-way ANOVAs, with treatment and trial block as factors. Multiple comparisons were made with Tukey's post hoc test when appropriate.

Supplementary Results

Experiment 1

Data from 8 rats with acceptable placements were included in the analysis. Rats used in the analysis required 10-42 days of training on the single trial Reference/Working memory task before achieving criterion performance, and another 3-13 days of training on the 5 trials/day version before receiving surgery. Upon re-training, they required an additional 4-12 days of training before receiving their first microinfusion test day.

Rats required 6.6±0.8 min (range 4.5-10.6 min) to complete the 5 trials following saline infusions. Following infusions of 25 ng bicuculline, rats required 8.0±0.9 min (range 4.8-17.8 min) to complete the session, and after 50 ng, they required 15.7±3 min (range 10.8-36.6 min).

Experiment 2

Data from 6 rats with acceptable placements were used in the analysis. Data from one rat with cannulae placed in the medial orbitofrontal cortex were excluded from the analysis. The remaining rats used in the analysis required 6-16 days of training on the single trial 8-arm foraging tasks before achieving criterion performance, and another 3-8 days of training on the 5 trials/day version before receiving surgery. They required 4-12 days of post-surgery re-training before the first microinfusion test day.

With respect to the amount of time required by rats to complete a test session, subjects required 6.4±0.3min (range 5.6-7.2 min) to complete the 5 trials following saline infusions. After treatment with the 50 ng dose of bicuculline, rats required 26.7±5 min (range 11.8-39.4 min).

Experiment 3

Data from 8 rats with acceptable placements were included in the analysis. Data from another 3 animals were excluded because of cannulae placements that were either ventral or anterior to the medial PFC. Rats required 8-20 days of training to achieve criterion performance on the single-trial version of task, and an additional 3-9 days to achieve a criterion the first trial in the massed-trials phase of training. Animals were re-trained for 3-9 days post-surgery before receiving counter-balanced infusions of saline or GABA receptor agonists.

Experiment 4

Data from 7 rats with acceptable placements were included in the analysis. Data from another 3 animals were excluded because of cannulae placements ventral to the medial PFC. Rats required 11 days of training before achieving criterion performance and then receiving infusions of bicuculline or saline.