Detection of the ocular dominance shift caused by monocular deprivation after GABAB receptor antagonist/agonist infusion in visual cortex

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Abstract

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Protocol

Minipump implantation

Step 1.

Animals were examined with an ophthalmoscope to ensure that they had no retinal disease or damage before each experiment. Details of our experimental methods have been described previously (Beaver et al., 2001, 2002). Briefly, animals were given acepromazine (0.1 mg/kg, i.m.), atropine (0.04 mg/kg, i.m.) and dexamethasone (1 mg/kg, i.m.), then anesthetized with ketamine (25 mg/kg, i.m.) and xylazine (1.5 mg/kg, i.m.), intubated, mounted in a stereotaxic apparatus and prepared for surgery following standard aseptic techniques. Anesthesia was maintained with halothane or isoflurane (0.5-1.2%) delivered in a 2:1 mixture of nitrous oxide and oxygen. The animal's core temperature was maintained at 38°C using a homeostatically controlled heating pad. The electrocardiogram, blood oxygen saturation and end-tidal carbon dioxide were continuously monitored. A small craniotomy (1.5 mm in diameter) was made over the left visual cortex 5 mm posterior to interaural zero and 2 mm lateral to midline. A hole was made in the dura and the tip of a 30 G cannula (Alzet brain infusion kit, Durect) was lowered into the cortex to a depth of 1.5-2 mm. The cannula was cemented in place and connected to an osmotic minipump (Alzet model 2001, Durect) containing one of the following solutions: vehicle alone (sterile 0.033 M phosphate-buffered saline, PBS), 20 mM baclofen in vehicle, or 20 mM SCH50911 in vehicle (drugs were obtained from Tocris Biosciences and Sigma). The minipump was inserted beneath the skin of the neck, the incision was sutured shut, and a topical lidocaine gel (2%) was applied to the incision site. After surgery, animals were given an antibiotic (Baytril, 2.5 mg/kg, i.m., once/day for three days) and an analgesic (Buprenex 0.01 mg/kg, i.m., twice/day for three days). Anesthesia and nitrous oxide were discontinued and the animal was allowed to recover on the heating pad. At the first sign of waking the animal was extubated, removed from the heating pad and observed until fully alert. The incision site was checked daily for any sign of opening or infection.

Monocular Deprivation

Step 2.

Early the next morning, about 12 hours after minipump implantation, the animal's right eye was sutured shut with 4-0 silk under halothane or isoflurane (0.5-3%) anesthesia. A small bead of ophthalmic antibiotic ointment (Neosporin or chlortetracycline hydrochloride) was applied to the eye before closing. The deprived eye was checked daily for any sign of opening or infection.

Electrophysiological recording

Step 3.

After four days of MD (4.5 days of drug infusion) in kittens or seven days of MD (7.5 days of drug infusion) in adults, animals were prepared for extracellular single unit recording. Surgical procedures incorporated the methods of Beaver et al. (2001), Shou et al. (1996), and/or An et al. (2014). Briefly, animals were given atropine (0.04 mg/kg, i.m.) and dexamethasone (1 mg/kg, i.m.), then anesthesia was induced with ketamine (25 mg/kg, i.m.), and a tracheotomy and venous catheterization were performed. All wounds and pressure points were treated with lidocaine (2%) and the animal was placed in a stereotaxic apparatus. Thereafter anesthesia was maintained with: pentobarbital (3 mg/kg/h, i.v.), or halothane (0.5–3%, in a 2:1 mixture of nitrous oxide and oxygen) in kittens; or urethane (given as an initial dose of 30 mg/kg, i.v., followed by the infusion of 20 mg/kg/h, i.v.) in adults. All animals were paralyzed with gallaminetriethiodide (8-10 mg/kg/h, i.v.) and placed on mechanical respiration. All intravenous solutions were delivered in a normal saline with glucose. Heart rate was monitored continuously as an indicator of anesthetic level. End-tidal carbon dioxide, blood oxygen saturation, and body temperature were monitored and maintained within normal physiological limits. The deprived eye was opened and 1-2 drops of phenylephrine (1%) and tropicamide (0.25%) were placed in each eye to retract the nictitating membrane and dilate the pupil. Both eyes were fitted with contact lenses to correct focus and prevent corneal desiccation.

The scalp was opened, the incision margins were treated with lidocaine (2%), and the minipump and cannula were removed and inspected to ensure that they were intact and functioning properly. A large craniotomy was made to expose the left primary visual cortex and the dura reflected to allow for six or more electrode penetrations. Epoxylite-insulated tungsten microelectrodes (3-5 MΩ, FHC Inc.) were advanced using a hydraulic micromanipulator (Narishige) angled at roughly 20° from vertical in an anterior to posterior direction to sample cells evenly across several OD columns.

The visual stimuli were moving sinusoidal gratings displayed on a CRT monitor (1024×768, 21 inch, 100 Hz, Sony) positioned 57 cm from the animal's eyes, covering 40×30 degrees of visual angle. The luminance non-linearity of the monitor was corrected by an inverse-gamma function applied with software. The mean luminance of the monitor was 60 cd/m^2 and environmental luminance on the cornea was near 0.1 lux. The program used to generate the stimuli was coded in MATLAB (Mathworks).

For each neuron the preferred spatial frequency, direction and temporal frequency were determined. In kittens, using the optimal stimulus parameters, cells were assigned to ocular dominance categories according to the seven-category scheme of Hubel and Wiesel (1962) based on the auditory discrimination of two independent listeners as described in previous studies (Beaver et al., 2001,2002). In adult cats, using the optimal stimulus parameters, the single unit activity evoked by the stimulation of each eye was recorded using an Igor program. To avoid sampling bias, recorded single units were separated by at least 200 µm along the electrode track. Six or more penetrations were made in the drug-infused hemisphere (contralateral to the deprived eye). Using the method of Beaver (2001), we compared drug treated neurons near the infusion site (1.0-3.5 mm distant) with unaffected neurons far from the infusion site (> 4.5 mm distant), providing an internal control for interanimal variability. After completing each penetration two or more electrolytic lesions were made

along the electrode track to facilitate anatomical reconstruction. At the end of the recording experiment, the animal was transcardially perfused with normal saline, followed by 4% paraformaldehyde in normal saline. The visual cortex was sectioned at 50 µm intervals and stained with cresyl violet. Penetrations were reconstructed to determine their distance from the infusion site and establish the laminar position of each recorded neuron.

Data Analysis

Step 4.

Response quality was assessed using an activity index in which the level of visually driven and spontaneous activity were each rated on a three-point scale (1 = low to 3 = high). The OD index for each cell is calculated as (peak RE – spontaneous RE)/[(peak LE – spontaneous LE) + (peak RE – spontaneous RE)], where LE and RE represent the response from the left eye and the right eye respectively. Using the seven-category scheme of Hubel and Wiesel (1962), a weighted ocular dominance (WOD) and binocularity index (BI) were then calculated as follows:

$$WOD=(1/6G_2 +2/6G_3+3/6G_4+4/6G_5+5/6G_6+G_7)/N$$

$$BI = [1/3(G_2+G_6)+2/3(G_3+G_5)+G_4]/N$$

With $G_{(i)}$ representing the number of cells in OD group i (i = 1-7; 1, deprived eye only and 7, non-deprived eye only) and N representing the total number of recorded cells (Kasamatsu et al., 1981). Thus, a WOD score of 1 indicates all cells respond only to the non-deprived eye, while a WOD score of 0 means all cells respond only to the deprived eye; and a BI score of 0 means that all cells are monocularly-driven, while a BI score of 1 means that all cells are binocularly-driven. For all measures data are expressed as mean ± SEM.

Statistical analysis was performed with GraphPad Prism 5 and MATLAB. Differences between OD histograms were assessed using a χ^2 test. Differences between two groups were assessed with *t*-test. Differences of WOD scores between near and far sites were evaluated with a paired *t*-test. Differences between three groups were evaluated with one-way ANOVA followed by Newman-Keuls post hoc test. Variation of WOD scores in different layers at near or far sites was evaluated with two-way ANOVA. Level of significance was $p < 0.05$.