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ENVIRONMENTAL ENRICHMENT DIFFERENTIALLY MODIFIES SPECIFIC COMPONENTS OF SENSORY-EVOKED ACTIVITY IN RAT BARREL CORTEX AS REVEALED BY SIMULTANEOUS ELECTROPHYSIOLOGICAL RECORDINGS AND OPTICAL IMAGING *IN VIVO*

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Abstract—Environmental enrichment of laboratory animals leads to multi-faceted changes to physiology, health and disease prognosis. An important and under-appreciated factor in enhancing cognition through environmental manipulation may be improved basic sensory function. Previous studies have highlighted changes in cortical sensory map plasticity but have used techniques such as electrophysiology, which suffer from poor spatial resolution, or optical imaging of intrinsic signals, which suffers from low temporal resolution. The current study attempts to overcome these limitations by combining voltage-sensitive dye imaging with somatosensory-evoked potential (SEP) recordings: the specific aim was to investigate sensory function in barrel cortex using multi-frequency whisker stimulation under urethane anaesthesia. Three groups of rats were used that each experienced a different level of behavioural or environmental enrichment. We found that enrichment increased all SEP response components subsequent to the initial thalamocortical input, but only when evoked by single stimuli; the thalamocortical component remained unchanged across all animal groups. The optical signal exhibited no changes in amplitude or latency between groups, resembling the thalamocortical component of the SEP response. Permanent and extensive changes to housing conditions conferred no further enhancement to sensory function above that produced by the milder enrichment of regular handling and behavioural testing, a finding with implications for improvements in animal welfare through practical changes to animal husbandry.

Key words: environmental enrichment, barrel cortex, somatosensory-evoked potentials, voltage-sensitive dye imaging, rodent, *in vivo*.

A crucial property of the CNS is its capacity to be modified as a consequence of experience, thereby providing the ability to adapt to an ever-changing environment. Since the 1940s investigations have described specific neuronal effects of environmental manipulations from the molecular level through to the behavioural level which have shed light on the possible mechanisms by which the environment may modify brain development, connectivity and function (Hebb, 1947; Bennett et al., 1964; Walsh and Cummins, 1975; van Praag et al., 2000). For example, anatomical changes such as growth of neuronal cell bodies, more extensive dendritic branching and improved vascularisation (Diamond et al., 1967; Greenough and Volkmar, 1973; Sirevaag et al., 1988; van Praag et al., 2000; Faherty et al., 2003; Nithianantharajah et al., 2004) are reflected in marked behavioural changes that include improvements in cognitive ability (Tang et al., 2001; Schrijver et al., 2002; Lee et al., 2003; Bruel-Jungerman et al., 2005; Leggio et al., 2005) and reduced levels of anxiety (Roy et al., 2001; Benaroya-Milshtein et al., 2004).

Some of the most basic, yet important, findings on the effects of environmental manipulations use sensory function as the key parameter, such as the contraction of the size of whisker and digit representations in primary sensory cortex (Coq and Xerri, 1998; Polley et al., 2004) that parallels improvements in behavioural performance (Bourgeon et al., 2004; Xerri et al., 2005). It has been suggested that by changing primary cortical processing circuitry, through which sensory information reaches higher-level processing centres, the perceptual context and behavioural performance of the animal is improved; an effect that becomes more apparent, the more demanding the behavioural task involved (Rosenzweig and Bennett, 1996; Li et al., 1998).

Previous investigations into environment-related sensory map plasticity, however, have used techniques that suffer from poor spatial or temporal resolution, as typified by electrophysiological approaches (Coq and Xerri, 1998) or optical imaging of intrinsic signals (Polley et al., 2004), respectively. The recording of local field potentials or unit activity fails to accurately capture spatiotemporal dynamics of cortical activation, yet such spatial patterns have been shown to be of great importance in sensory processing, motor performance and cognition (Nicolelis et al., 1997; Harris, 2005; Krueger et al., 2008; Shirvalkar, 2009). On the other hand, optical imaging of intrinsic signals, which

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Abbreviations: Di-4-ANEPPS, pyridinium 4-[2-[6-(dibutylamino)-2-naphthalenyl]-ethenyl]-1-(3-sulfopropyl)hydroxide; ECG, electrocardiogram; EEG, electroencephalogram; Max-E, maximally-enriched; Min-E, minimally-enriched; Non-E, non-enriched; ROI, region of interest; SEP, somatosensory-evoked potential; VSDI, voltage-sensitive dye imaging.

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does capture spatial activation patterns, only visualises activity indirectly via signals related to cortical metabolism that are aggregates of excitatory and inhibitory activity of the preceding 1–2 s (Grinvald et al., 1999).

Problems with spatial and temporal resolution were overcome in the current study by combining voltage-sensitive dye imaging (VSDI) with concurrent somatosensory-evoked potential (SEP) recordings; the optical imaging approach of VSDI can detect changes in membrane potential in real-time (Grinvald and Hildesheim, 2004). The current study emulated previous investigations and evaluated the impact of environmental enrichment on sensory-evoked activity within the rodent barrel cortex (Fox, 2002; Frostig, 2006; Holtmaat and Svoboda, 2009). We used this combination of techniques to profile the effects of an enriched environment on the function of sensory cortex as accurately as possible. Since "enrichment" can encompass a wide range of different experiences and levels of stimulation, we exposed rats to different types of environment in order to establish what might be the critical factors.

EXPERIMENTAL PROCEDURES

Animals

Male Fischer-344 rats (F344/NHsd; $n=31$) were used and divided into three experimental groups: non-enriched (Non-E), minimally-enriched (Min-E) and maximally-enriched (Max-E). Animals in the Min-E and Max-E groups were supplied by Harlan (Bicester, UK) at 28-days of age ($n=12$ in each group) whereas animals in the Non-E group were supplied at 12 months of age ($n=7$) and only housed briefly before being used in the VSDI/SEP study. As many countries now standardise husbandry and housing conditions by way of governmental codes of practice (e.g. in the UK: Animal (Scientific Procedures) Act, 1986 and the "Code of Practice for the Housing and Care of Animals in Designated Breeding and Supplying Establishments"; http://www.homeoffice.gov.uk/science-research/animal-research; accessed May 19, 2010), basic neurophysiology of the Non-E group can be considered to broadly resemble that of a typical rodents obtained from such a facility. Both minimally-enriched and maximally-enriched groups experienced identical behavioural testing and handling over the course of the investigation but differed in their cage contents: minimally-enriched cages contained standard bedding/nesting substrates and rat houses whereas maximally-enriched cages were much larger and contained additional regularly-changing items including toys, platforms and manipulanda. In all environments, animals were kept on a 12-h dark/light cycle at a temperature of 22 °C with food (RM3P, Special Diets Services Ltd., UK) and water available *ad libitum*.

The experiments described were approved by the local University ethical committee and all procedures were performed with Home Office approval under the Animals (Scientific Procedures) Act 1986. After 49 days, and while continuing to be housed in their respective environments, the Min-E and Max-E animals underwent training in behavioural tasks described below. Animals in the Non-E group underwent no behavioural testing or handling and were kept for between 1 and 3 weeks before being used in the VSDI/SEP stage of the study.

Housing environments and experiences

The minimally-enriched environment consisted of a plastic cage of size $76\times60\times30$ cm^3, the contents of which were equivalent to standard laboratory housing conditions and contained sawdust, bedding and cardboard houses (380 sq. cm floor space per animal; >2.5 times minimal UK Home Office guidelines based on maximal body weight of 250 g). The enriched environment consisted of a wood/wire-mesh cage of size $90\times60\times60$ cm^3; the lower portion of the walls (height=27 cm) consisted of wooden panels while the remaining portion and the ceiling were comprised of wire-mesh. The contents were chosen to provide high levels of sensory, motor and cognitive stimulation and changed twice per week to maintain novelty but were carefully selected to avoid inadvertent harm to the animals (Bayne, 2005). An inner platform increased the overall floor space to 7764 sq. cm (total of 647 sq. cm per animal; >4.3 times UK Home Office guidelines). Both enriched and control environments were cleaned every 2 weeks, during which time animals were held in standard laboratory cages; animals from both groups were also weighed and identification marks re-applied to their tails at these times. Throughout all stages of the study, handling of animals was always matched between Min-E and Max-E groups.

A range of behavioural evaluations were performed on the Min-E and Max-E groups that are being prepared in a separate publication: whisker sensory-discrimination (Guic-Robles et al., 1989, 1992); open-field (Walsh and Cummins, 1976; Elliott and Grunberg, 2005); hyponeophagia (Dulawa and Hen, 2005); Barnes maze (Barnes, 1979; Pompl et al., 1999); novel object recognition (Prickaerts et al., 2002, 2005). These tests began 2 months following supply of animals and involved a total of 69 days of handling and/or behavioural testing and were completed 7 months later.

Surgical procedures

After Non-E animals habituated to their environment for 1 week and behavioural testing was completed in Min-E and Max-E groups (at which time all rats were between 12 and 13 months old), rats were anaesthetised with urethane (1.15 g/kg) and chloral hydrate (0.16 g/kg) and transferred to a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). All drugs were obtained from Sigma-Aldrich (Poole, UK). Respiratory and cardiovascular parameters were recorded throughout surgical and experimental procedures. Rate and depth of respiration were recorded using a custom-built monitor based around an accelerometer integrated circuit (Devonshire et al., 2009). Heart rate was recorded via single-strand copper electrocardiogram (ECG) recording leads inserted s.c. behind each forelimb and connected to a custom-built ECG-processing unit. A craniotomy was performed over the primary somatosensory cortex at the following approximate stereotaxic co-ordinates: anterior-posterior=1–5 mm, medial-lateral=4–8 mm. A single trepanne hole (~1 mm Ø) was drilled in the left frontal bone into which a short loop-tipped silver wire electrode (0.2 mm Ø; Intracel, Royston, UK) was inserted to act as a reference electrode. An imaging chamber comprising a 3 mm section of a disposable 5 ml syringe (Becton-Dickinson, Oxford, UK) was cemented in place around the craniotomy using dental cement (Duralay, Reliance Dental; Worth, IL, USA).

Sensory stimulation

All whiskers on the left-hand side of the snout were trimmed except for whisker C2. The remaining whisker was stimulated 3 mm from its base by a 26G hypodermic needle attached to a piezoelectric wafer (PL122.11, Physik Instrumente; Harpenden, UK). Displacement of the wafer was produced by applying an electrical potential across it (10v for 20 ms) to give approximately 2 mm of movement in a caudal direction. Whiskers were deflected for 1 s at 2 and 10 Hz in a randomly interleaved pattern; trials were separated by 60 s and a total of 144 trials were presented to each animal over a period of 4 h.