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Effect of Enriched Environment on Ca^{2+} Uptake via NMDA Receptors Into Barrel Cortex Slices of Spontaneously Hypertensive Rats

Molupe Lehohla,[1] Vivienne Ann Russell,[1,2] and Lauriston Kellaway[1]

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Exposure to an enriched environment provides animals with informal learning opportunities and is associated with increases in brain size, cortical thickness, neuron size, dendritic branching, spine density, and number of synapses per neuron. The NMDA receptor is involved in synaptic plasticity. This study sought to determine the effect of exposure to an enriched environment on NMDA receptor function in barrel cortex slices of spontaneously hypertensive rats (SHR) and their control Wistar-Kyoto (WKY) rats. An assortment of items such as PVC pipes, metal pipes, metal boxes, metal ladders, and a polystyrene maze, were placed successively in the cages of test animals to create an enriched environment. After 2 weeks, the rats were killed. Their brains were rapidly removed, cooled in continuously oxygenated HEPES buffer (pH 7.4), and sliced in a vibratome to produce 0.35-mm thick slices. The barrel cortex was dissected from slices corresponding to 8.6–4.8 mm anterior to the interaural line and incubated with $^{45}Ca^{2+}$ and 100 μM NMDA for 2 min. There was no difference between rats exposed to an enriched environment and rats kept in standard cages. Enrichment of environment did not alter NMDA-stimulated Ca^{2+} uptake into barrel cortex of SHR and WKY.

Key words: Glutamate; receptor; function; hyperactive; attention; ADHD.

INTRODUCTION

Tactile information acquired through the vibrissae is of great behavioral significance to nocturnal animals such as rats and other rodents. The vibrissae are linked to somatosensory cortex of the rat brain by a network of neurons that pass through the thalamic "relay nuclei" en route to the cortex (Woosley and Van der Loos, 1970). The primary targets of thalamic afferents are clusters of spiny stellate neurons and star pyramidal cells in layer 4 of the somatosensory cortex called barrels (Feldmeyer *et al.*, 1999). Each cortical barrel represents a whisker on the contralateral side of the face (Woosley and Van der Loos, 1970). The barrel cortex is the most highly organized part of the somatosensory cortex. These cells receive whisker-specific-thalamic input and distribute their output mainly within the cortical column (Feldmeyer *et al.*, 1999). Synaptically connected spiny stellate neurons within layer 4 have been suggested to function as a cortical amplifier of incoming thalamic signals (Egger

[1]Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.
[2]To whom correspondence should be addressed at Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Observatory, 7925, Cape Town, South Africa. E-mail: russell@curie.uct.ac.za

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et al., 1999). The majority of neurons that connect the barrel cortex to other parts of the brain use glutamate as a neurotransmitter (Keller, 1995).

Two subtypes of glutamate receptors occur in the somatosensory cortex namely the fast acting ligand-gated ionotropic receptors and slow acting G-protein coupled metabotropic receptors (Zigmond *et al.*, 1999). The ligand-gated receptors are divided into NMDA and non-NMDA receptor subtypes according to their pharmacology. High densities of NMDA receptors have been demonstrated in the supragranular layer of the rat primary somatosensory cortex and in layer 5 (Jaarsma *et al.*, 1991). In layer 4 high NMDA receptor densities were specifically confined to the barrel hollows (Jaarsma *et al.*, 1991). The distribution of NMDA receptors in the barrel field showed a strong coincidence with the zone of termination of the specific sensory afferents from the ventral posterior nucleus of the dorsal thalamus (Jaarsma *et al.*, 1991).

It has been suggested that the NMDA receptor channel is involved in synaptic plasticity during development (McDonald and Johnston, 1990), which refers to the ability of the brain to change its structure and function (Kolb and Whishaw, 1998). Experience is the major stimulant of brain plasticity in animal species as diverse as insects and humans (Kolb and Whishaw, 1998). There are large neuronal changes associated with experience. These include increases in brain size, cortical thickness, neuron size, dendritic branching, spine density, synapses per neuron, and glial numbers (Kolb and Whishaw, 1998). Experience can either be in the form of exposure to an enriched environment or task-specific training (Kolb and Whishaw, 1998). Exposure to an enriched environment provides animals with informal learning opportunities (Rosenzweig and Bennett, 1996) and is an important model of plastic changes in the brain (Filipkowski *et al.*, 2000). An enriched environment is described as a combination of complex inanimate and social stimulation and is enriched in relation to standard laboratory housing conditions (Van Praag *et al.*, 2000). The environment is complex and the novel objects as well as their location are changed frequently to provide variation over a period of time (Van Praag *et al.*, 2000). It is widely accepted that rats use their vibrissae to actively explore their environment. It could be argued that by enriching the environment there could be additional stimulation of the barrel cortex and that this may be reflected by plastic changes involving the NMDA receptor. Since spontaneously hypertensive rats (SHR) by their very nature are more active than their control Wistar-Kyoto rats (WKY) in standard laboratory cages, exposure to an enriched environment may have an even greater effect on SHR barrel cortex. This study therefore sought to determine the effect of exposure to an enriched environment on NMDA-stimulated Ca^{2+} uptake into barrel cortex slices of WKY and to compare these findings to SHR.

MATERIALS AND METHODS

Chemicals and Reagents

All reagents were obtained from Merck, Germany, except HEPES and NMDA, which were obtained from Sigma, St. Louis, MO, USA $^{45}Ca^{2+}$ from NEN Life Science, Boston; scintillation fluid from Zinsser Analytic, Berkshire, UK; and BSA from Miles Laboratories, South Africa.

Animals

Thirteen 3-week-old male SHR and ten 3-week-old male WKY were used after approval was obtained from the University of Cape Town Animal Ethics Committee. The two strains of animals were further subdivided into two groups one of which was exposed to an enriched environment consisting of a succession of PVC pipes, metal pipes, metal boxes, and metal ladders while the other group remained in the standard rat cage. There were two sets of each of the objects, one used for SHR and the other for WKY. The novel items were introduced to the cages at the same time each day and replaced after 24 h. After 2 weeks, on the night preceding the experiment the rat to be sacrificed was transferred to a new cage in which the novel environment was a self-made polystyrene maze for 16 h. All groups of rats were exposed to a normal 12-h light–dark cycle and allowed free access to commercial pellets and tap water.

Procedure

On the day of the experiment, 5- to 6-week-old rats were killed by cervical dislocation and decapitated between 9:30 and 10:30. The brain was rapidly removed, cooled in a continuously oxygenated, ice-cold incubation HEPES buffer (120 mM NaCl, 1 mM $MgCl_2$, 3.36 mM KCl, 10 mM D-Glucose, 20 mM HEPES buffer (pH 7.4), 1.2 mM $CaCl_2$), and sliced coronally using a vibratome (slice thickness = 0.35 mm). The entire process including the slicing procedure took about 40 min. Optimal procedures for obtaining viable slice preparations from brain were followed (Aitken *et al.*, 1995; Lipton *et al.*, 1995). The somatosensory cortex was dissected from slices corresponding to anteroposterior coordinates 8.70–4.84 mm (Paxinos and Watson, 1986) with reference to the interaural line. The somatosensory cortex was arbitrarily divided into three divisions (rostral, middle, and caudal), each division having four slices, to establish if there was regional variation. The slices were then subjected to an assay consisting of a 45-min equilibration phase at room temperature followed by a 5-min preincubation phase in HEPES buffer at 35°C. This was followed by a 2-min incubation in HEPES buffer containing 1 μCi of $^{45}Ca^{2+}$ at 35°C. NMDA-stimulated calcium uptake into each region was measured according to the method described previously (Feldman *et al.*, 1990; Lehohla *et al.*, 2000, 2001). Since previous studies showed that there were no inter hemispheric differences in NMDA-stimulated Ca^{2+} uptake (Lehola *et al.*, 2000), barrel cortex slices from the left hemisphere were used as controls while slices from the right hemisphere were used as tests in each of the three divisions of the barrel cortex. The effect of NMDA on $^{45}Ca^{2+}$ uptake was determined by incubating test slices in buffer containing NMDA (100 μM). These slices were compared to control slices that were incubated in buffer that did not contain NMDA. After incubation, the slices were subjected to a series of washes (10-s wash, followed by a 15-min wash) in tubes containing 10-mL HEPES buffer with $CaCl_2$ replaced by 10 mM lanthanum chloride ($LaCl_3$). The slices were then transferred to flat petri dishes containing 10-mL HEPES buffer with $CaCl_2$ replaced by 10 mM $LaCl_3$ in order to facilitate retrieval of the slices, for a third and final wash to remove any excess $^{45}Ca^{2+}$ from the slices. After washing, the slices were sonicated for 3 min in an ultrasound sonicator (cycle time = 2 s) to rupture the cells and release $^{45}Ca^{2+}$ taken up. Part

of the sonicate was counted in a liquid scintillation counter while the remainder was used for protein determination. The method employed was Miller's modification of the Lowry method (Miller, 1959) using bovine serum albumin as a standard.

Statistical Analysis

Statistica was used to analyze the Ca^{2+} uptake data by means of a two-way analysis of variance (ANOVA) with main factors, strain (WKY, SHR) and environment (standard, enriched). A probability of $P < 0.05$ was considered significant. The results are expressed as nanomoles $^{45}Ca^{2+}$/mg of protein/2 min. and are presented as mean ± SEM.

RESULTS

A two-way ANOVA revealed that there was no significant effect of "strain" or "environment" on total, basal, or NMDA-stimulated Ca^{2+} uptake into barrel cortex slices.

There was no significant difference in total Ca^{2+} uptake into the entire barrel cortex of WKY and SHR exposed to an enriched environment (10.2 ± 0.543 nmol Ca^{2+}/mg protein, $n = 5$ and 8.6 ± 0.222 nmol Ca^{2+}/mg protein, $n = 6$, respectively, Fig. 1) compared to total uptake into the barrel cortex of WKY and SHR reared in a standard rat cage (9.5 ± 0.732 nmol Ca^{2+}/mg protein, $n = 5$ and 9.0 ± 0.892 nmol Ca^{2+}/mg protein, $n = 7$, respectively, Fig. 1). Basal uptake into the entire barrel cortex of WKY and SHR exposed to an

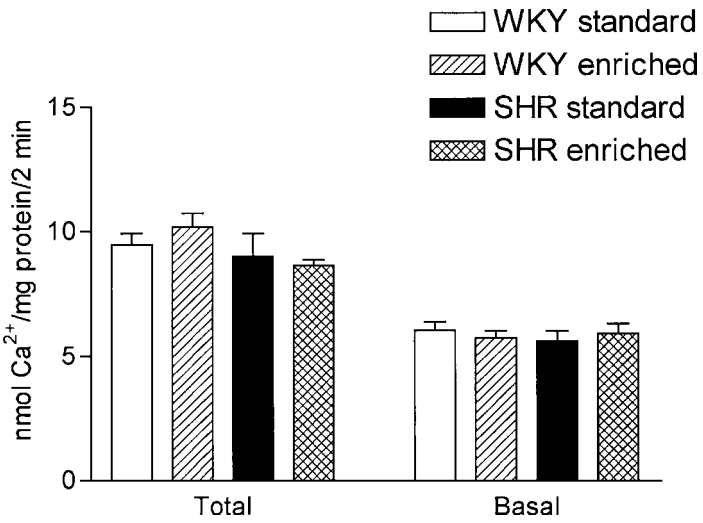

Figure 1. Average total and basal uptake of Ca^{2+} into barrel cortex slices of WKY and SHR reared in an enriched environment compared to WKY and SHR reared in the standard laboratory cages. Results are mean ± SEM nmol Ca^{2+}/mg protein/2 min ($n = 5$–7).

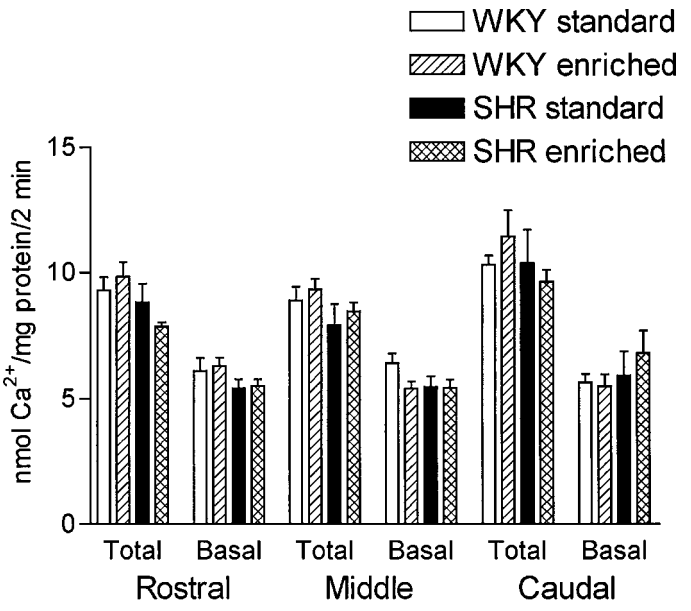

Figure 2. Total and basal uptake of Ca^{2+} into rostral, middle, and caudal divisions of barrel cortex slices of WKY and SHR reared in an enriched environment compared to WKY and SHR reared in standard cages. Results are mean ± SEM nmol Ca^{2+}/mg protein/2 min ($n = 5$–7).

enriched environment (5.7 ± 0.303 nmol Ca^{2+}/mg protein, $n = 5$ and 5.9 ± 0.405 nmol Ca^{2+}/mg protein, $n = 6$, respectively, Fig. 1) was not significantly different from basal uptake into the entire barrel cortex of WKY and SHR housed in a standard cage (6.0 ± 0.336 nmol Ca^{2+}/mg protein, $n = 5$ and 5.6 ± 0.425 nmol Ca^{2+}/mg protein, $n = 7$, respectively, Fig. 1) for 2 weeks.

Total uptake (Fig. 2) into the rostral, middle, and caudal divisions of the barrel cortex of WKY and SHR rats exposed to an enriched environment was not different from total uptake into the corresponding regions of WKY and SHR housed in a standard environment. Basal uptake into the rostral, middle, and caudal regions of WKY and SHR exposed to an enriched environment was not different from basal uptake into the corresponding regions of WKY and SHR reared in a standard environment.

NMDA-stimulated uptake of Ca^{2+} into the entire barrel cortex of WKY and SHR exposed to an enriched environment (4.5 ± 0.508 nmol Ca^{2+}/mg protein, $n = 5$ and 2.8 ± 0.536 nmol Ca^{2+}/mg protein, $n = 6$, respectively) was not significantly different from NMDA-stimulated Ca^{2+} uptake into entire barrel cortex of WKY and SHR reared in a standard environment (3.5 ± 0.335 nmol Ca^{2+}/mg protein, $n = 5$ and 3.4 ± 0.519 nmol Ca^{2+}/mg protein, $n = 7$, Fig. 3).

There was no significant difference between NMDA-stimulated Ca^{2+} uptake into the rostral, middle, and caudal divisions of the barrel cortex of WKY and SHR exposed to an enriched environment and NMDA-stimulated Ca^{2+} uptake into corresponding regions of WKY and SHR reared in a standard environment (Fig. 4).

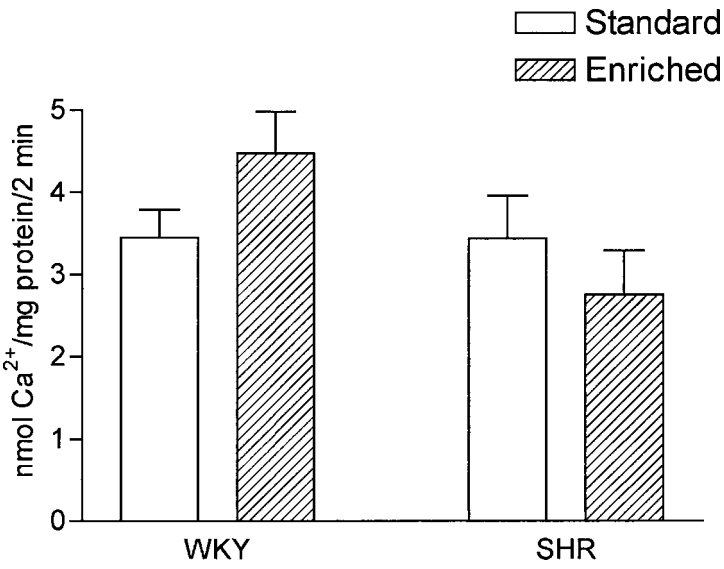

Figure 3. Average NMDA-stimulated uptake of Ca^{2+} into barrel cortex slices of WKY and SHR reared in an enriched environment compared to WKY and SHR reared in standard cages. Results are mean ± SEM nmol Ca^{2+}/mg protein/2 min ($n = 5–7$).

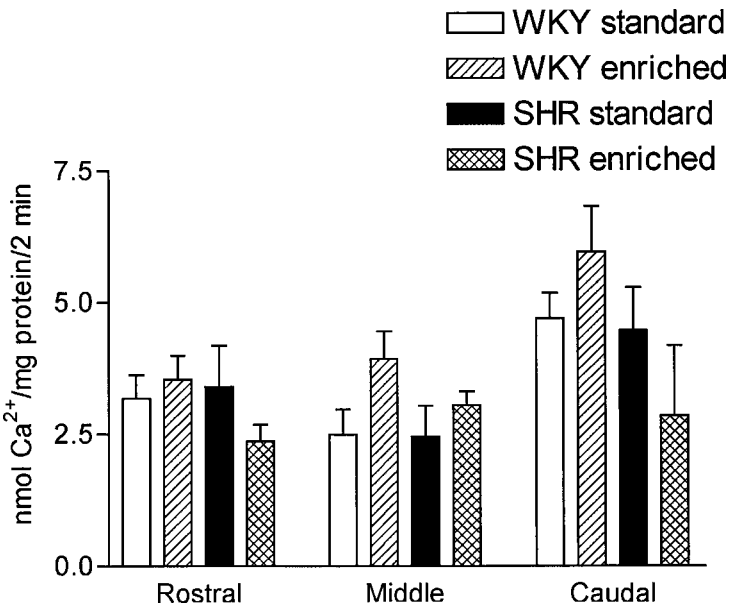

Figure 4. NMDA-stimulated uptake of Ca^{2+} into rostral, middle, and caudal divisions of barrel cortex slices of WKY and SHR reared in an enriched environment compared to WKY and SHR reared in standard cages. Results are mean ± SEM nmol Ca^{2+}/mg protein/2 min ($n = 5–7$).

DISCUSSION

Exposure of both SHR and WKY rats to an enriched environment in this study did not have an effect on NMDA receptor function. In a similar study, Por *et al.* (1982) showed that there was no difference in neurotransmitter receptors between rats exposed to an enriched environment and controls, despite having found a 5% increase in cortical weights of animals exposed to an enriched environment compared to their littermates in the normal cage. Por *et al.* (1982) exposed rats to an enriched environment for 35–55 days. Using receptor binding assays, they found no evidence that differential rearing environments caused significant differences in dopamine, serotonin, α or β adrenergic, muscarinic cholinergic, or GABA receptor levels in rat cortical membrane preparations (Por *et al.*, 1982). Also Bardo and Hammer (1991), using autoradiography showed that dopamine D1 and D2 receptor densities in the nucleus accumbens were not different between groups of rats reared in the enriched environment for 30 days and rats reared in a standard environment.

It has previously been shown that exposure to an enriched environment for different time periods leads to increases in brain weights (Rosenzweig *et al.*, 1968) and an increase in acetylcholine esterase (AchE) and cholinesterase (ChE) activity (Rosenzweig *et al.*, 1968). Volkmar and Greenough (1972) used Golgi staining to show increased higher order branching in layers 2, 4, and 5 pyramidal stellate cells in the rat visual cortex after exposure to an enriched environment for 29–31 days. These results showed that the density of dendritic spines and extent of dendritic arborization in the rat cortex are environmentally regulated to a certain extent (Volkmar and Greenough, 1972). It was thought that the number and density of dendritic spines and size of receptor areas observed in rats exposed to an enriched environment might reflect a difference in the number of cortical neurotransmitter binding sites (Por *et al.*, 1982). It was for this reason too, that the present study was undertaken in an attempt to determine whether plastic changes involving NMDA receptors occur in rat barrel cortex. The present results do not support the hypothesis that exposure to an enriched environment will increase the number and function of NMDA receptors in rat barrel cortex. Despite their inherent increased activity and attention to novel stimuli, SHR also did not demonstrate enhanced NMDA receptor function in the barrel cortex or any of its subfields studied.

Increased expression of immediate-early genes was suggested to be a consequence of increased intracellular Ca^{2+} concentration (Morgan and Curran, 1989) which in turn is a consequence of neuronal activity, specifically glutamate activation of NMDA receptors (Steiner and Gerfen, 1994). Increased expression of immediate-early genes *c-fos* and *c-jun* in the rat barrel cortex as a result of exposure to an enriched environment has been shown by Filipkowski *et al.* (2000) and Staiger *et al.* (2000). It has been argued that changes in the levels of expression of immediate-early genes can reflect changes in neuronal activity (Sagar *et al.*, 1988). As a result of increased neuronal activity during exploration of the enriched environment one would have expected to measure increased Ca^{2+} uptake in response to stimulation of NMDA receptors but that was not the case in this study. Exposure to the enriched environment did not increase NMDA receptor function in barrel cortex of SHR or WKY. Differences between the two studies include the fact that in the present study, the animals were exposed to different, novel, stimuli in the enriched environment for a period of 24 h for 2 weeks compared to the 20-min exposure/day used by Filipkowski *et al.* (2000), where the whiskers were directly stimulated. On the other hand Staiger *et al.* (2000) exposed

the animals to an enriched environment overnight before experimenting on them and got the same result as Filipkowski *et al.* (2000). This rules out the possibility that duration of exposure to an enriched environment is critical in this study. This is further supported by the fact that Rosenzweig *et al.* (1968) found a similar increase in brain weight and AchE activity in three groups of animals. One group was exposed to an enriched environment for 2.5 h day, and spent the rest of the day in the normal cage. Another group was exposed to the an enriched environment for 4.5 h day while another group was exposed to a novel environment for 24 h. Thus a few hours of daily exposure to an enriched environment increased brain weights as much as 24-h exposure (Rosenzweig *et al.*, 1968). In the present study the animals were exposed to an enriched environment for 2 weeks but this did not alter NMDA receptor function in rat somatosensory cortex.

CONCLUSION

Total, basal, and NMDA-stimulated $^{45}Ca^{2+}$ uptake into barrel cortex of SHR and WKY exposed to an enriched environment was not significantly different from SHR and WKY reared in a normal environment.

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