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VISUAL DEPTH PERCEPTION IN NORMAL AND DEPRIVED RATS: EFFECTS OF ENVIRONMENTAL ENRICHMENT

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Abstract—A proper maturation of stereoscopic functions requires binocular visual experience and early disruption of sensory-driven activity can result in long-term or even permanent visual function impairment. Amblyopia is one paradigmatic case of visual system disorder, with early conditions of functional imbalance between the two eyes leading to severe deficits of visual acuity and depth-perception abilities. In parallel to the reduction of neural plasticity levels, the brain potential for functional recovery declines with age. Recent evidence has challenged this traditional view and experimental paradigms enhancing experience-dependent plasticity in the adult brain have been described. Here, we show that environmental enrichment (EE), a condition of increased cognitive and sensory-motor stimulation, restores experience-dependent plasticity of stereoscopic perception in response to sensory deprivation well after the end of the critical period and reinstates depth-perception abilities of adult amblyopic animals in the range of normal values. Our results encourage efforts in the clinical application of paradigms based on EE as an intervention strategy for treating amblyopia in adulthood.

Key words: depth perception, stereopsis, visual cliff, environmental enrichment, amblyopia, monocular deprivation.

INTRODUCTION

Depth perception, which refers to the visual system ability to see the world in three dimensions starting from a two-dimensional representation on the retina, relies on both monocular (MON) and binocular (BIN) cues. Stereopsis is the main BIN cue allowing depth to be judged through the computation of slight differences between the images received by each eye (BIN disparities; Cumming and DeAngelis, 2001). The maturation of stereoscopic depth perception occurs during postnatal life with a relatively rapid time course (e.g., Bauer, 1973; Held et al., 1980; Timney, 1981; Birch et al., 2005) coinciding with the maturation of cortical neurons tuned for BIN disparities (Pettigrew, 1974; Freeman and Ohzawa, 1992; Chino et al., 1997).

A large number of studies in animal models highlighted how dramatically early sensory deprivation can affect stereoscopic abilities and visual cortex physiology. A total absence of patterned visual experience, indeed, leads to the degradation of stereoscopic perception, probably due to the lack of a precise tuning for BIN disparities in visual cortical neurons (e.g., Wilson and Riesen, 1966; Pettigrew, 1974; Tees, 1974; Kaye et al., 1981). Similarly, early disruption of concordant BIN visual experience owing to conditions of visual deprivation, such as strabismus, anisometropia or MON occlusion, has been shown to deteriorate stereoscopic depth skills (e.g., Blake and Hirsch, 1975; Timney, 1983; Crawford et al., 1993; Mitchell et al., 2009; Levi et al., 2011).

The essential role of experience in driving the proper maturation of stereoscopic functions is particularly evident within a restricted time window in early postnatal life, the so-called critical period (CP), during which brain circuits display a high sensitivity to acquire instructive and adaptive signals from the external environment. No sign of alteration of stereoacuity thresholds can be detected in adult subjects exposed to an anomalous BIN experience (Timney, 1983, 1990; Fawcett et al., 2005).

The high potential for plasticity during the CP may also favour the onset of developmental pathological states due to an anomalous perturbation of sensory-driven activity. A paradigmatic case is that of amblyopia, a widely diffused pathology of the visual system, deriving from conditions of early functional imbalance between the two eyes (Mittelman, 2003) and characterized by a dramatic degradation of visual acuity and perceptual deficits affecting stereoscopic depth perception (Holmes and Clarke, 2006; Levi, 2006).

Due to the age-dependent decline of plasticity within the brain, amblyopia treatment is effective only when starting early in life (Holmes and Clarke, 2006). Recent studies, however, have challenged this view, providing evidence that intervention strategies enhancing neural plasticity in adulthood may allow recovery from amblyopia well after

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Abbreviations: ANOVA, analysis of variance; BIN, binocular; CI, choice index; CP, critical period; DD, differential depth; DI, discrimination index; EE, environmental enrichment; MD, monocular deprivation; MON, monocular; RS, reverse-sutured; SC, standard conditions; TDM, total distance moved; V1, primary visual cortex.

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the closure of the CP (for review, Bavelier et al., 2010; Baroncelli et al., 2011a). Among them, environmental enrichment (EE), an experimental paradigm increasing multi-sensory and cognitive stimulation, physical activity and social interactions (Sale et al., 2009; Baroncelli et al., 2010a), emerged as a very promising approach. We demonstrated that EE reactivates plasticity processes in the adult visual cortex, promoting both a marked ocular dominance shift of cortical neurons in response to MON deprivation (MD; Baroncelli et al., 2010b) and a full recovery of binocularity and visual acuity in adult amblyopic animals (Sale et al., 2007; Baroncelli et al., 2012; Tognini et al., 2012).

It remains unknown, however, whether enhancing brain plasticity through EE can influence visual depth perceptual abilities. This issue is particular relevant in amblyopia, since in animal models a rescue of stereopsis has been proved harder to achieve than that of visual acuity (Timney, 1983; Mitchell et al., 1994). Here, we employed the visual cliff task to explore the effects of EE on stereoscopic abilities in animals either subjected to sensory deprivation in adulthood or rendered amblyopic through long-term MON occlusion.

EXPERIMENTAL PROCEDURES

Animal housing

A total of 118 Long-Evans rats were used in this study, conducted in accordance with protocols approved by Italian Ministry of Public Health, and in conformity with the European Communities Council Directives. Animals were maintained at a temperature of 20–22 °C under a 12-h light/dark cycle and had *ad libitum* access to food and water. Environmental enrichment consisted of a large cage (100 × 50 × 82 cm) with two or more floors linked by stairs, containing several food hoppers, running wheels and differently shaped objects, which were repositioned and/or substituted with others once a week. Every cage housed 6–8 adult rats. Standard housing consisted of a standard cage (40 × 30 × 20 cm), housing a maximum of three adult rats.

Animal treatment

Animals were housed in standard conditions (SC) until adulthood. At the postnatal day (P) 60 they were assigned to either the enriched environment group (EE) or to the SC group for 3 weeks. After 2 weeks, rats were anesthetized with avertin (2% solution of 2,2,2-tribromoethanol, 200 mg/kg) and MD was performed through eyelid suturing. Subjects with even minimal spontaneous re-opening were excluded from the study. Another group of animals were rendered amblyopic by long-term MD starting from the CP (P21) and reverse-sutured (RS) at P60. After 3 weeks of differential rearing, animals were tested in the visual cliff apparatus. The day before the visual cliff test BIN conditions were reinstated (i.e., the occluded eye was re-opened using thin scissors) in all experimental groups, except for rats tested in MON conditions. Great care was taken to prevent eye inflammation or infection through the topical application of antibiotic and cortisone.

Visual cliff task

A modification of the visual cliff proposed by Booher and Walk (1968) was employed. The apparatus consisted of a rectangular arena (100 × 40 cm^2) constructed in poly(vinyl chloride) with black walls and bordered by black curtains to prevent the animal's escape. The arena was divided into two 50 × 40 cm^2 Plexiglas plates. A moving platform, the depth of which could be varied by means of a mechanical scissor jack, was placed below each glass plate. A patterned floor consisting in 3-cm black and white checked photographic paper covered the platform's surface (see Fig. 1). Incandescent lamps placed below the two patterned floors illuminate both surfaces to equate the brightness of the two sides. A telecamera was hanging on the apparatus and was connected to a computer by which the experimenter could observe and record the rat's behaviour. Testing took place in a quiet room. Assignment to either exploration or descent test was made randomly. *Exploration test.* The arena was divided into a shallow and a deep side. At the shallow side the patterned floor was positioned immediately below the glass plate, while at the deep side the checked platform was moved to 29 cm below the glass plate (Fig. 1a). Each animal was placed on the shallow side and the total time the rat spent exploring each side of the arena was automatically recorded by the Noldus Ethovision system. The trial ended after 5 min. The exploration path and velocity (VEL) of each animal were also recorded and analysed with the Noldus Ethovision system. The arena was cleaned between trials with an alcohol solution. A discrimination index (DI) was calculated as follows: $(t_s - t_d)/t_{tot}$, where t_s and t_d are, respectively, the time spent exploring the shallow side and the deep side of the arena, and t_{tot} is the total time of the test procedure. Each animal was tested only once. *Descent test.* An elevated board (height: 10 cm) was placed in correspondence of the arena midline at the border between the shallow and the deep side. A modification of the procedure described by Tees (1974) was employed. To avoid confounding effects deriving from tactile stimulation (see Lore et al., 1967), at the shallow side the patterned floor was positioned 5 cm below the board basement and Plexiglas plates were removed (Fig. 1b). Animals must first be conditioned to distinguish a high differential depth (DD) with great reliability before the limit of this ability can be assessed. There are three phases to the task: pre-training shaping, task training and testing. In the pre-training phase, animals were familiarized with the arena for 10 min. After a two-day pre-exposure to Fonzies, a highly palatable food containing cheese powder, animals were mildly food deprived and trained to descend onto the shallow platform. Each animal was placed at one end of the starting board and was allowed 3 min to descend; a correct choice was rewarded with a bit of Fonzies (given to the animal by the experimenter using tweezers), while after errors, ''no-choices'' and fallings the animal was simply put back into the cage. During the training phase, the checked platform at the deep side was positioned 24 cm lower than that at the shallow side. The position of the deep side with respect to the starting board was changed randomly. After animals have achieved near-perfect (80% or more) performance over 20–40 trials on a pseudorandom schedule in the training phase, the testing phase could begin. The DD between the two sides was made progressively smaller (24, 16, 8 cm). Each rat was tested four times for each depth. The surfaces of both sides and the starting board were cleaned after each animal was tested. Each animal was tested in a single viewing condition. The experimenter observed the rat behaviour through the computer, to prevent possible bias in the animal's response. A preference for the shallow or the deep side was defined as placement of the four paws on one side within 3 min. Trials on which rats fell off the board were recorded as falls in the data analysis. The actions of rats that failed to respond within the 3-min time were scored as ''no-go'' responses. A choice index (CI) for each DD was calculated as the difference between the number of correct choices and errors (''no-go'' responses were scored as 0), divided by the total number of trials without falling $[(d_s - d_d)/(4 - f)$, where d_s is the number of descents to the shallow side, d_d is the number of descents to the deep side and f is the