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Research report

Down-regulation of hippocampal BDNF and Arc associated with improvement in aversive spatial memory performance in socially isolated rats

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ABSTRACT

Rats deprived of social contact with other rats at a young age experience a form of prolonged stress that leads to long-lasting changes in behavioral profile. Such isolation is thought to be anxiogenic for these normally gregarious animals, and the abnormal reactivity of isolated rats to environmental stimuli is thought to be a product of prolonged stress. We now show that isolation of rats at weaning reduced immobility time in the forced swim test, decreased sucrose intake and preference, and down-regulated both brain-derived neurotrophic factor (BDNF) and activity-regulated cytoskeletal associated protein (Arc) in the hippocampus. In the Morris water maze, isolated rats showed a reduced latency to reach the hidden platform during training, indicative of an improved learning performance, compared with group-housed rats. The cumulative search error during place training trials indicated a reliable difference between isolated and group-housed rats on days 4 and 5. The probe trial revealed a significant decrease of the average proximity to the target location in the isolated rats suggesting an improvement in spatial memory.

Isolated rats also showed an increase in the plasma level of corticosterone on the 5th day of training and increased expression of BDNF and Arc in the hippocampus on both days 1 and 5. These results show that social isolation from weaning in rats results in development of depressive-like behavior but has a positive effect on spatial learning, supporting the existence of a facilitating effect of stress on cognitive function.

1. Introduction

Early adverse experiences in humans are associated with an increased risk for development of psychiatric disorders such as anxiety and major depression [29]. Rearing of rodents from weaning to adulthood in isolation induces various behavioral [30,51,63,70,76,77] and neurochemical [36,38,63,64,65,67,70] alterations in comparison with group-housed controls, suggesting that socially isolated rodents represent an animal model of neuropsychiatric disorders [22].

Although many studies have examined the effects of social isolation in rodents on depression-like symptoms as well as on molecular and neurochemical markers related to major depression, the results are often conflicting as a consequence of differences in species or strains studied as well as in isolation timing and implemented procedures. For instance, the percentage immobility time of animals in the forced swim test [54] has been found to be unchanged [30], decreased [28], or increased [8,18] by social isolation, and sucrose consumption or preference (a measure of anhedonia) was found to be decreased after isolation in adulthood [72] or increased in animals isolated as adolescents [8,26,27]. Several studies have described a reduced level of serotonergic and noradrenergic function and metabolism in various brain regions of isolated animals [22,26].

Neurotrophic factors, in particular brain-derived neurotrophic factor (BDNF), have been implicated in the regulation of mood disorders and antidepressant effects, leading to formulation of the neurotrophic hypothesis of depression [11–13,21]. BDNF is also implicated in the pathophysiology of anxiety disorders [42]. Preclinical studies have shown that chronic stress down-regulates the expression of BDNF in the hippocampus [22]. BDNF is also thought to play an important role in long-term potentiation (LTP), a form of synaptic plasticity that is thought to underlie the formation of long-term memory [5,32,45]. BDNF induces the expression of

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many genes in hippocampal cells, with one of the most prominently affected genes being that for activity-regulated cytoskeletal associated protein, or Arc [1,78]. Arc is the product of an immediate-early gene involved in regulation of actin cytoskeletal dynamics underlying consolidation of LTP and contributes to activity-dependent synaptic plasticity and adaptive behavior such as memory storage. It has also been indirectly implicated in modulation of cellular functions that are perturbed in depressive states, several studies have reported upregulation of Arc in forebrain structure following antidepressant treatment [37,40,47]. Given that its expression in the dendrites of neurons is modulated by synaptic activity, Arc has been proposed as a neuronal marker [68].

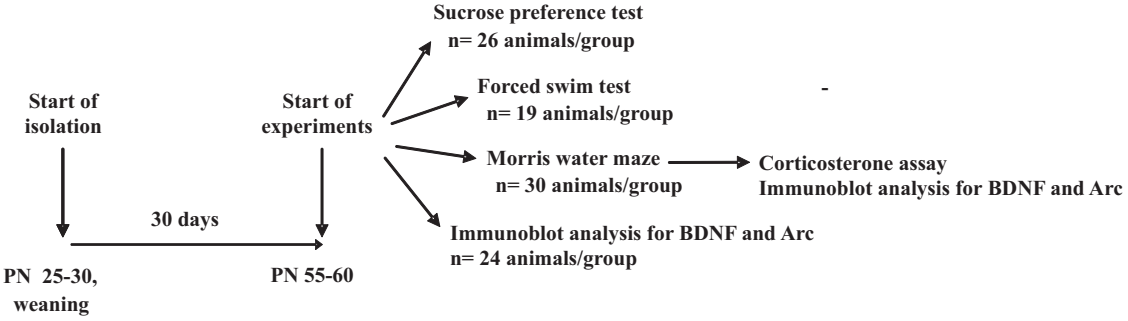
On the basis of these evidences, we have now investigated in rats the effects of social isolation from weaning on depressive behavior and on the expression of BDNF and Arc in the hippocampus.

Extensive evidence indicates that stress hormones epinephrine and glucocorticoids are critically involved in memory consolidation of emotionally arousing experiences [9,58,59]. Several studies have examined the effect of social isolation in rodents on the acquisition and retention of spatial learning in the Morris water maze, but the results have again been conflicting because of differences in the species or strains of animals studied and in the isolation protocols used [31,36,62,76]. Recently, has been shown that systemic corticosterone administration increased the expression of hippocampal protein Arc and enhance long-term memory in the inhibitory avoidance [44]. Since learning in the Morris water maze is an aversive motivated behavior and therefore constitutes a stressful event and the steroidogenic response to a novel stress is enhanced in isolated rats [63], an increased secretion of corticosterone may result in cognitive performances improvement. Thus, spatial learning and memory were also examined with the Morris water maze, and the plasma concentration of corticosterone and hippocampal expression of BDNF and Arc were measured at various stages of training.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley CD rats at 25–30 days of age, immediately after weaning, were housed for 30 days either in groups of six to eight per cage (59 cm × 38 cm × 20 cm) or individually in smaller cages (42 cm × 26 cm × 15 cm). They were maintained on an artificial 12-h-light, 12-h-dark cycle at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were freely available at all times. Separate groups of rats were used in each of the experiments. The isolated animals were left undisturbed in their cages and received the minimal handling associated with fortnightly husbandry. All the experiments were performed after the isolation period (postnatal day 55–60). Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).



2.2. Sucrose preference test

Rats isolated or group-housed for 30 days were allowed access for 48 h to two bottles, one containing sucrose solution (32%, w/v) and the other tap water. The positions of the two bottles were changed every 12 h to control for position preference. Fluid (sucrose solution or water) consumption was calculated by weighing each bottle before and after animal exposure. Sucrose preference was calculated as: 100% × sucrose solution consumption (g)/total fluid consumption (g).

2.3. Forced swim test

The forced swim test is a 2-day procedure in which rats swim under conditions in which escape is not possible. On the 1st day (pre-test), a rat was placed in a Plexiglas cylinder (40 cm in height, 20 cm in diameter) that was filled to a height of 25 cm with water at 25 °C and placed in a dimly lit room. After 15 min, the rat was removed from the water, dried with a towel, and transferred to a heated enclosure. Twenty-four hours later, the animal was placed in the cylinder again for 5 min (test). Both the pre-test and test sessions were videotaped from the side of the cylinder, and animal behavior was digitized with the use of the Ethovision system (Noldus, Wageningen, The Netherlands). The durations of climbing (defined as upward movement of the forepaws aimed toward the sides of the cylinder), swimming (defined as horizontal movement throughout the cylinder), and immobility (defined as minimum movements required to maintain the head above water in the absence of the other two behaviors) were manually scored from the video by two independent observers, blind to the experimental groups.

2.4. Morris water maze

The Morris water maze consisted of a circular pool (150 cm in diameter, 60 cm in depth) whose interior was painted black. It was located in the center of a room dedicated to measurement of this behavioral paradigm. The water temperature was maintained at 25 ± 2 °C with the use of a submersible digital water-heating system. The pool was divided into four quadrants, and a removable circular escape platform (10 cm in diameter, 32 cm in height) was introduced into one of the quadrants (target quadrant) at a depth of 2 cm below the surface of the water. Each rat was subjected to four training trials on each of five consecutive days (by first placement into the pool in the quadrant next to the target quadrant). After the animal had climbed onto the platform it was allowed to remain there for 15 s before the next trial; if it had not found the escape platform at the end of 120 s, it was gently guided to the platform and allowed to rest there for 15 s. The time elapsed (latency) before the animal climbed onto the platform, swim speed, and distance travelled during each trial were recorded; animals that did not climb onto the platform before the end of the trial period were assigned a latency value of 120 s. At 24 h after the last training trial, each rat was subjected to a probe trial, in which the escape platform was removed from the pool and the animal was released from the quadrant opposite to the original platform location and allowed to swim freely for 60 s.

Behavioral data from the training and probe tests were acquired and analyzed using an automated tracking system (Ethovision, Noldus Wageningen, The Netherlands). Using this software, the precise mouse location (in *x*, *y* coordinates) was recorded throughout the probe test (capture rate 10 frames/s). From this spatial distribution, proximity measure [23] was calculated automatically.

2.5. Immunoblot analysis

The hippocampus was homogenized in a solution of 2% sodium dodecyl sulfate. Protein samples (40 μg in 15 μl) were incubated for 5 min at 95 °C before fractionation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 12% Bis–Tris Criterion XT Pre-cast 26 W gels, Bio-Rad). The separated proteins were transferred to a polyvinylidene difluoride membrane with a Criterion™ Blotter (Bio-Rad), 100 V for 1 h (transfer buffer: Trizma-base 190 mM, glycine 25 mM, methanol 20%, v/v) and subjected to immunoblot analysis with rabbit polyclonal antibodies to BDNF (1:200 dilution, Santa Cruz Biotechnology) or with mouse monoclonal antibodies to Arc (1:250 dilution, Santa Cruz Biotechnology). The membrane was incubated with primary antibodies overnight at 4 °C, and immune complexes were detected with horseradish peroxidase–conjugated secondary antibodies and chemiluminescence reagents (ECL, Amersham Biosciences). The amounts of BDNF and Arc were quantified by analysis of the corresponding bands on the autoradiogram with a densitometer (GS-700, Bio-Rad). Data were normalized by dividing the optical density of the bands corresponding to BDNF or Arc by that of the band for glyceraldehyde-3-phosphate dehydrogenase (GAPDH, housekeeper protein), which was revealed by reprobing the membrane with mouse monoclonal antibodies to this protein (Millipore).