# Remodeling of Hippocampal Spine Synapses in the Rat Learned Helplessness Model of Depression

Tibor Hajszan, Antonia Dow, Jennifer L. Warner-Schmidt, Klara Szigeti-Buck, Nermin L. Sallam, Arpad Parducz, Csaba Leranth, and Ronald S. Duman

**Background:** Although it has been postulated for many years that depression is associated with loss of synapses, primarily in the hippocampus, and that antidepressants facilitate synapse growth, we still lack ultrastructural evidence that changes in depressive behavior are indeed correlated with structural synaptic modifications.

**Methods:** We analyzed hippocampal spine synapses of male rats ($n = 127$) with electron microscopic stereology in association with performance in the learned helplessness paradigm.

**Results:** Inescapable footshock (IES) caused an acute and persistent loss of spine synapses in each of CA1, CA3, and dentate gyrus, which was associated with a severe escape deficit in learned helplessness. On the other hand, IES elicited no significant synaptic alterations in motor cortex. A single injection of corticosterone reproduced both the hippocampal synaptic changes and the behavioral responses induced by IES. Treatment of IES-exposed animals for 6 days with desipramine reversed both the hippocampal spine synapse loss and the escape deficit in learned helplessness. We noted, however, that desipramine failed to restore the number of CA1 spine synapses to nonstressed levels, which was associated with a minor escape deficit compared with nonstressed control rats. Shorter, 1-day or 3-day desipramine treatments, however, had neither synaptic nor behavioral effects.

**Conclusions:** These results indicate that changes in depressive behavior are associated with remarkable remodeling of hippocampal spine synapses at the ultrastructural level. Because spine synapse loss contributes to hippocampal dysfunction, this cellular mechanism may be an important component in the neurobiology of stress-related disorders such as depression.

**Key Words:** Corticosterone, depression, desipramine, electron microscopic stereology, synaptic plasticity, stress

Depression is a devastating illness with an estimated lifetime prevalence of 17% in the United States (1). It is a leading cause of disability worldwide, bringing about considerable loss of life by suicide (2), as well as being a risk factor for cardiovascular disease (3) and a multitude of neurological disorders, including dementia (4,5). Despite intensive research on the neurobiology of depression and mechanisms of antidepressant action, current clinical management of the disease remains limited (6).

Evidence for hippocampal atrophy in depressed patients (7–9), as well as derailment of many hippocampus-related functions in depression (10–12) indicates that the hippocampus is critically involved in the disease (13). It has been postulated for many years that hippocampal dysfunction in depression is related to loss of dendritic spine synapses of principal cells (14–16). The spine synapse is the most abundant and a highly plastic form of synapses in the hippocampus; it is considered a unit of information processing and represents a point of connectivity between principal neurons (17). Previous studies have confirmed that hippocampal spine synapse remodeling indeed occurs in response to stress and depression (18,19). However, there are many conflicting reports regarding the nature, hippocampal distribution, and functional significance of these synaptic changes, which may be explained by the fact that most of the earlier studies have used indirect, light microscopic approaches to analyze synaptic plasticity.

To address these issues, here we use electron microscopy, a technique that provides sufficient resolution to visualize synaptic specializations, to investigate hippocampal spine synapses in association with behavioral changes in the rat learned helplessness (LH) model of depression and antidepressant response.

## Methods and Materials

Adult male Sprague-Dawley rats ($n = 127$, 200–250 g; Charles River Laboratories, Wilmington, Massachusetts) were kept under standard laboratory conditions. The animals were group housed and maintained on a 12/12-hour light/dark cycle, with tap water and regular rodent chow available ad libitum. All animal protocols used in this study were approved by the Institutional Animal Care and Use Committee of Yale University School of Medicine.

### Learned Helplessness

A standard LH paradigm was used, as we published previously (20,21). In this paradigm, an initial exposure to uncontrollable stress rapidly and profoundly disrupts the ability to acquire escape responses when animals are later placed in a situation in which stress is escapable. The behavioral dysfunction usually reaches a level where stressed animals do not even make an effort to escape the shock. In the case of rats, this escape deficit is reduced by subchronic (5–7 days) (21), but not acute, treatment with conventional antidepressants, such as fluoxetine or desipramine. Without treatment, however, the escape deficit may last up to several weeks. Testing was conducted in commercial shuttle boxes (Med Associates, St. Albans, Vermont) divided into two equal compartments by a central barrier. A computer-operated guillotine door built into the central barrier provided

From the Departments of Obstetrics, Gynecology, and Reproductive Sciences (TH, KS-B, NLS, CL), Psychiatry (AD, JLW-S, RSD), Pharmacology (KS-B, RSD), and Neurobiology (CL), Yale University School of Medicine, New Haven, Connecticut; and Institute of Biophysics (TH, AP), Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary.

Address reprint requests to Tibor Hajszan, M.D., Ph.D., Department of OBGYN, Yale University School of Medicine, 333 Cedar St LSOG205C, New Haven, CT 06520; E-mail: tibor.hajszan@yale.edu.

Received May 2, 2008; revised September 4, 2008; accepted September 30, 2008.

0006-3223/09/$36.00
doi:10.1016/j.biopsych.2008.09.031

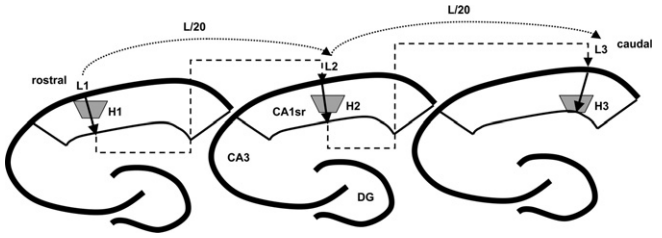


**Figure 1.** Schematic drawing illustrating our sampling scheme for electron microscopic stereology. We used a two-dimensional coordinate system with length (L) and height (H) axes. Using the same group of sections that underwent volume estimation previously, the length of CA1 stratum pyramidale was measured in each section and combined to create the L axis. Then, the L axis was divided by the number of desired sampling sites (L/20), and a random number (N) between 0 and L/20 was selected. The coordinate of the first sampling site on the L axis (L1) was localized at N μm, and the subsequent coordinates at L/20 μm apart (L2–L3, etc.), going along the L axis (the CA1 stratum pyramidale) in the direction of CA3 > subiculum and from rostral to caudal (dotted arrows). Subsequently, the height of CA1 stratum radiatum (CA1sr) was measured at each L axis sampling coordinate, along lines drawn perpendicular to stratum pyramidale (bold arrows). These 20 height measurements were then combined to create the H axis. Sampling sites along the H axis (H1–H3, etc.) were localized using the same method as described for the L axis above, going along the height measurement lines in the direction of stratum pyramidale > stratum lacunosum-moleculare and from rostral to caudal (dashed arrows). Shaded trapezoids demonstrate the approximate locations of tissue samples. This technique was repeated to localize sampling sites in the remaining sampling areas. CA3 stratum pyramidale, dentate gyrus stratum granulosum, and the brain surface represented L axes for CA3, dentate gyrus (DG), and motor cortex, respectively.

passage between compartments. Scrambled shocks were delivered to the grid floor consisting of stainless steel rods (5 mm in diameter) placed 1.7 cm apart center-to-center. Inescapable footshock (IES) was administered in one side of shuttle boxes with the guillotine door closed (60 scrambled footshocks, .85 mA intensity, 15 sec average duration, 60 sec average intershock interval). This number and severity of IES presentation has been shown to be effective in inducing helpless behavior in rats (20,21). Nonstressed control animals were exposed to the chambers but did not receive footshock (NS treatment). Helpless behavior was evaluated by analyzing performance in an active escape paradigm. Active escape testing consisted of 30 trials of escapable footshock (.65 mA intensity, 35 sec maximum duration, 90 sec average intertrial interval), with the guillotine door being opened at the time of shock onset. An initial 5 fixed ratio 1 trials, during which one shuttle crossing terminated the shock, was followed by 25 trials when animals had to cross from one side of the shuttle box to the other, and then return, to terminate shock (fixed ratio 2 trials). Shock was terminated automatically if the response requirement was not met within 35 sec. Escape latencies were recorded automatically for each trial by a computer. Escape latency represented the time it took for animals to escape shock, while in the case of escape failures, escape latency was set at 35 sec. Inescapable footshock administration and active escape testing were done in a dimly lit room between 1000 and 1600 hours.

### Electron Microscopic Stereology

The number of spine synapses in CA1 stratum radiatum (CA1sr), CA3 stratum lucidum and radiatum (CA3sl/sr), dentate gyrus stratum moleculare (DGsm), as well as in layer II/III of motor cortex (MII/III) was calculated as published previously (22). Animals were perfusion fixed through the ascending aorta with a mixture of 4% paraformaldehyde and .1% glutaraldehyde dissolved in phosphate buffer (.1 mol/L, pH 7.4). Brains were dissected out and postfixed overnight in the same fixative without glutaraldehyde. Throughout both the hippocampus and the motor cortex, 100-μm thick serial sections were cut in the coronal plane using a vibratome and systematically sorted into 10 groups. Because there is a considerable overlap between the two structures, we analyzed the right-side hippocampus together with the left-side motor cortex and vice versa, the selection being randomized within each treatment group. One randomly selected group of sections from both structures was postfixed in 1% osmium tetroxide (40 min), dehydrated in ethanol (the 70% ethanol contained 1% uranyl acetate; 40 min), and flat-embedded in Durcupan (Electron Microscopy Sciences, Fort Washington, Pennsylvania) between slides and coverslips. Using these embedded sections, the volume of the sampling areas (CA1sr, CA3sl/sr, DGsm, and MII/III) was estimated utilizing the Cavalieri Estimator module of the Stereo Investigator system (MicroBrightField, Villiston, Vermont) mounted on a Zeiss Axioplan 2 light microscope (Carl Zeiss MicroImaging, Inc., Thornwood, New York).

Thereafter, 20 sampling sites for electron microscopic analysis were localized in each sampling area using a systematic random approach, as modified from MacLusky *et al.* (22) and illustrated in Figure 1. Blocks were assembled for ultracutting and trimmed and approximately four 75-nm thick consecutive ultrasections were cut at each identified sampling site using a Reichert Ultracut E ultrotome. At each sampling site, digitized electron micrographs (Figure 2) were taken for the physical disector in a Tecnai-12 transmission electron microscope (FEI Company, Hillsboro, Oregon) furnished with a Hamamatsu HR/HR-B CCD camera system (Hamamatsu Photonics, Hamamatsu, Japan), at a final magnification of 11,000x. The disector technique requires picture pairs depicting identical regions in adjacent ultrasections, these identical regions being identified by landmarks, such as

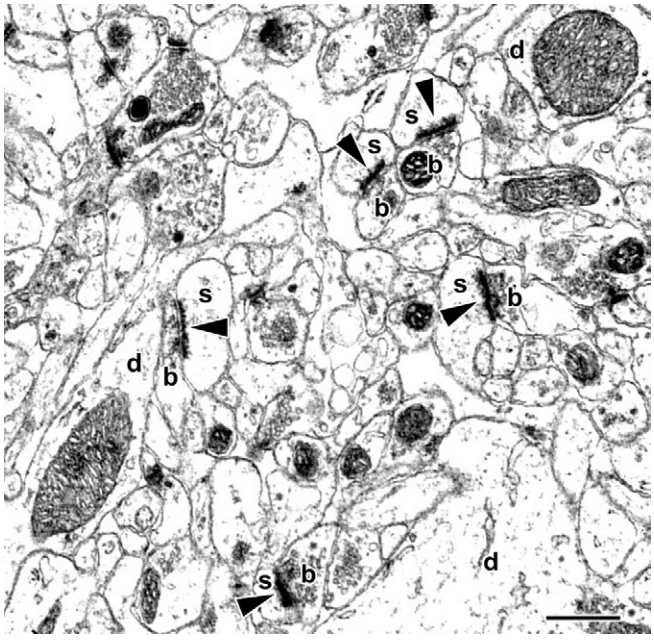


**Figure 2.** High-power representative electron micrograph depicting the CA1 stratum radiatum of a stressed desipramine-treated rat. Arrowheads point to examples of spine synapses. Scale bar, 500 nm. b, boutons; d, dendritic shafts; s, spines.