Supporting Information

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SI Text

SI Methods

Cognitive assessment. Rats were trained on the spatial delayed alternation task in a T maze; successful performance of this task relies on the medial prefrontal cortex [Larsen and Divac (1978) Selective ablations within the prefrontal cortex of the rat and performance of delayed alternation *Physiol Psychol* 6:15–17]. Training commenced when rats were three months old, and rats were no more than six months old at the completion of the study. After achieving a two-day (two-test session) mean of 68–78% correct (baseline, BL), rats were separated into four groups with equivalent mean BL performance. Rats were treated with either the PKC inhibitor CHEL or vehicle (veh). Groups were as follows: no-stress+veh, $n = 8$; no-stress+CHEL, $n = 8$; stress+veh, $n = 7$; stress+CHEL, $n = 8$. Separate groups were trained on a spatial discrimination task before chronic stress (no-stress, $n = 6$; stress, $n = 6$).

Training and testing were conducted within the same 2-h time frame by a blinded experimenter. Food rewards were miniature chocolate chips. For the spatial delayed alternation task, rats were rewarded for entering either arm of the T maze on the first trial. Thereafter, for a total of 12 trials per session, rats were rewarded only if they entered the maze arm that was not previously chosen. Thus, the animal had to maintain the previous selection "online" over a delay to guide the next choice. Four delay lengths (2, 5, 10, and 15 seconds) were quasirandomly distributed over the 12 trials to prevent ceiling effects. Consecutive errors were defined as the highest number of consecutive entries into one choice arm in a test session. For the spatial discrimination task, rats were rewarded for entering one arm (right or left). Groups were counterbalanced for arm assignment. The spatial discrimination task requires similar motivational, locomotor, and spatial abilities as delayed alternation, but does not require an intact prefrontal cortex (44). For both tasks, the choice point of the maze was wiped with alcohol between trials to remove any olfactory clues.

Morphological Analyses

Criteria for inclusion of layer II/III prelimbic PFC neurons in the study. In order for a neuron to be included in the analysis, it had to satisfy the following criteria: (*i*) have a cell body located within layer II/III of the prelimbic region [Paxinos G, Watson C (1998) *The Rat Brain in Stereotaxic Coordinates* (Academic, London), 4th Ed.] as defined by cytoarchitectural characteristics; (*ii*) demonstrate complete filling of dendritic tree, as evidenced by well-defined endings and dark and consistent filling; (*iii*) demonstrate intact primary and secondary branches; (*iv*) have an apical extent of at least 300 μm and a secondary branch emanating from the apical trunk between 20–70 μm from the soma; and (*v*) have regions for spine quantification unobscured by neighboring branches.

Neuronal Locations for Spine Counting. Spines were quantified in five locations in each reconstructed neuron: (*i*) apical branch(es) lying 200 μm from the soma (30-μm segment, mean density was obtained if more than one branch met this criterion); (*ii*) the first apical branch longer than 30 μm emanating from the apical trunk 20–70 μm from the soma (the first 30-μm segment of this branch); (*iii*) apical trunk (first 50-μm segment from edge of soma); (*iv*) proximal basal branch (first 30-μm segment from edge of soma); and (*v*) distal basal branch (30–60 μm the edge of the soma). All protrusions that were in direct continuity with the dendritic shaft, or spine heads located within 1.5 μm from the dendritic shaft, were identified as a spine. Spine density was expressed as number of spines per μm of dendrite.

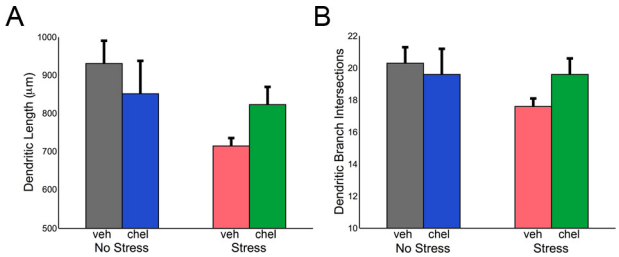

Fig. S1. The effects of the PKC inhibitor, chelerythrine (CHEL), compared to vehicle (veh) on the dendritic retraction induced by chronic stress exposure. Daily PKC inhibition results in a modest, but not statistically significant, restoration of stress-induced atrophy of the apical dendrite in layer II/III pyramidal cells. (*A* and *B*) Total dendritic length (*A*) and number of branch intersections (*B*) were determined by summing the output of sholl analysis up to 300 μm in radial distance from the soma (see Fig. 3*E*). Results represent mean +SEM.