Leverpress Escape/Avoidance Training Increases Neurotrophin Levels in Rat Brain

DAVID S. ALBECK,[1] KEVIN D. BECK,[2,3] LING-HSUAN KUNG,[1] KAZUHIRO SANO,[1] AND FRANCIS X. BRENNAN[4,5]

[1]*Department of Psychology, University of Colorado at Denver, Campus Box 173, P.O. Box 173364, Denver CO 80217-3364*
[2]*VA Medical Center, East Orange, NJ 07018*
[3]*Department of Neuroscience, New Jersey Medical School, UMDNJ, Newark, NJ 07018*
[4]*VA Medical Center, Philadelphia PA, 19104*
[5]*Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia PA 19104*

In addition to their well-known role in neural development, the neurotrophins BDNF and NGF help mediate the plasticity that occurs in the brain to promote learning. Exposure to learning procedures often leads to increases in neurotrophins, while exposure to stress often results in decreases. It is unclear how the neurotrophins would respond to an aversive learning task. Therefore, BDNF and NGF content in the dorsal striatum, hippocampus, and basal forebrain was measured following discrete trial lever-press escape/avoidance conditioning. Conditioning significantly increased levels of both neurotrophins in hippocampus and basal forebrain, relative to home cage controls (HCC). Contrary to expectations, the dorsal striatum did not show any significant changes. However, significant correlations were observed between dorsal striatal neurotrophins and aspects of avoidance performance. This may indicate that the dorsal striatum is involved in the performance aspects of the task. Results are discussed in terms of the role of neurotrophins in the acquisition of new information, and the neural structures involved in different types of memory.

Introduction

A RECENT THEORY OF MEMORY processing in the brain proposes there are three distinct memory systems operating in parallel, each responsible for recording different aspects of experience (White & McDonald, 2002). These systems are identified by their most prominent structure: the hippocampus, the dorsal striatum, and the amygdala. The hippocampal system is postulated to encode working memory, the dorsal striatum stimulus-response formation, while the amygdala is involved with emotional memory (White & McDonald, 2002).

Address for Correspondence: David Albeck, Department of Psychology, University of Colorado at Denver, Campus Box 173, P.O. Box 173364, Denver, Colorado 80217-3364. E-mail: dalbeck@carbon.cudenver.edu

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Discrete trial lever press escape/avoidance conditioning (E/A) is an operant task used to study aversive conditioning in rats (Brennan, Beck, & Servatius, 2003) and mice (Brennan, 2004). This paradigm requires the animal to press a lever to either terminate, or avoid entirely, an aversive footshock stimulus. Although the brain structures responsible for this type of learning are currently not well understood, it appears likely that the dorsal striatal system is important, since E/A requires formation of a stimulus-response relationship.

The neurotrophins Brain-Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF) are important for synaptic plasticity (Schinder & Poo, 2000), in particular long-term-potentiation (LTP; Kelly, Conroy, & Lynch, 1998; Kovalchuk et al., 2002), a putative mechanism for memory formation at the synaptic level. Both BDNF (Hall, Thomas, & Everitt, 2000) and NGF (Woolf et al., 2001) levels in the hippocampus increase with contextual learning. Since some amount of contextual learning is likely occurring during the E/A paradigm, BDNF and NGF levels were measured in the hippocampus, dorsal striatum as well as another brain region involved with the hippocampal memory system, the basal forebrain (White & McDonald, 2002). We hypothesized that both neurotrophins would be elevated in the dorsal striatum, because of its presumed role in the acquisition of the response.

Methods

Subjects

Subjects were 16 male Sprague-Dawley rats from Charles River (Kingston, NY) that were group housed (two to three per cage), and given *ad lib* access to food and water, except during the escape/avoidance (E/A) session. They were maintained on a 12/12-light/dark cycle with lights on at 0700. All cage mates were in the same experimental group and training was performed during the light phase.

Conditioning Procedure

All procedures were approved by the IACUC of the Philadelphia VA Medical Center, and were similar to those described previously (Brennan, 2004). Briefly, E/A sessions were conducted in operant chambers equipped with ABET software (Lafayette Instruments, Lafayette, IN). Chambers had a lever on one wall, a house-light and a speaker on the opposite wall to deliver the 1000-Hz warning signal (WS). Trials began with the WS and house-light being activated. If the rat did not make a leverpress after 1 minute of the WS, a 1-sec, 1.0-mA footshock was delivered and the WS remained on. Shocks occurred on the average of one per minute until a leverpress response. The shock, WS, and house-light were all terminated by a leverpress and a 6-min safety period began. The safety period was signaled by a flashing light located immediately above the lever (Brennan, Beck, & Servatius, 2003). Subjects received a "free" escape if no response had occurred in 20 minutes. Leverpresses after the shock had begun were classified as "escapes," while responses during the initial 1-min of the WS before the shock were classified "avoidances." Training consisted of a single 4-hr session.

Brain Dissections

All subjects were briefly exposed to CO_2 and sacrificed 1-hr after the E/A session. Brains were quickly removed, target regions dissected and frozen on dry ice until analysis. Target brain regions were: whole hippocampus, basal forebrain (Coordinates from Paxinos & Watson, (1998): Anterior/

posterior +1.0 mm to −0.26 mm Bregma, ventral to corpus callosum, medial to lateral ventricles), and dorsal striatum (top half of striatum using corpus callosum as dorsal border, lateral ventricle as medial border and external capsule as lateral border, from +2.2 mm to +1.0 mm Bregma anterior/posterior).

Assay Procedures

BDNF and NGF protein was measured using ELISA kits (Promega Corp., Madison, WI, USA) according to kit instructions (as in Albeck et al., 2003).

Statistics

The number of escape and avoidance responses by hour, across the conditioning session, as well as number of leverpresses during the safety period, a measure of anxiety (Berger & Starzec, 1988) were analyzed via repeated measure ANOVAs. All data are presented as mean ± standard error of the mean (SEM).

Results

Behavioral

In general, the avoidance performance was poor relative to other cohorts (Brennan, Beck, & Servatius, 2003) although subjects did generally acquire the leverpress response. The average number of escape responses per hour across the session decreased significantly $F(3,21) = 6.55$, $p < 0.01$, Figure 1, left side, while the mean numbers of avoidance responses across session are plotted in

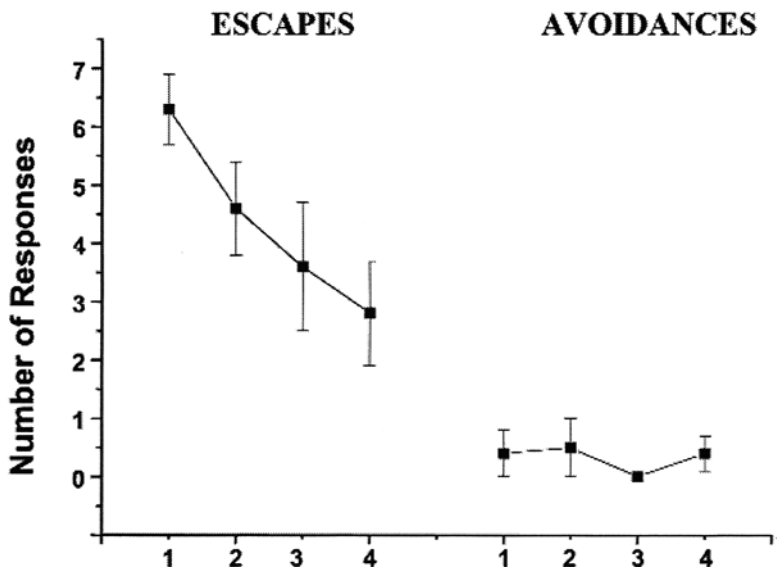

FIG. 1. Left Panel: Number of Escape responses across the four-hour session. The average number of escape responses per hour decreased significantly ($p < 0.05$, *) from hour 1 to hour 3 of conditioning and from hour 1 to hour 4. Right Panel: Number of Avoidance responses across the four-hour session.

Figure 1, right side. The majority of subjects did not make any avoidance responses, precluding a significant effect $F(3,21) < 1.0$. Finally, subjects decreased their leverpresses during the safety periods, $F(3,21) = 5.55, p < 0.01$, indicating a decrease in anxiety.

Neurotrophin Levels

BDNF and NGF were analyzed via one-way ANOVA (Trained vs. Controls) for each brain region. BDNF values are presented in Figure 2, while the NGF values are presented in Figure 3. In the hippocampus, E/A conditioning increased both BDNF, $F(1,14) = 5.56, p < 0.05$ and NGF, $F(1, 14) = 4.85, p < 0.05$ levels versus controls. The E/A animals showed a trend toward higher BDNF in the basal forebrain, $F(1,14) = 4.05, p = 0.06$, and E/A rats had significantly higher NGF than controls $F(1,14) = 4.68, p < 0.05$. BDNF levels in the basal forebrain positively correlated with both the total number of responses during the fourth hour ($r = 0.76, p < 0.05$), and the total number of presses during the safety periods in the fourth hour ($r = 0.75, p < 0.05$). In the dorsal striatum E/A training did not significantly alter BDNF $F(1,14) = 1.03, p > 0.05$, or NGF, $F(1,14) < 1.0$. However, NGF levels did negatively correlate with both the total number of avoidance responses ($r = -0.75, p < 0.05$), and the number of avoidance responses during the fourth hour ($r = -0.75, p < 0.05$).

Discussion

The current experiment revealed that exposure to a leverpress E/A procedure increased levels of BDNF and NGF in both hippocampus and basal forebrain. Contrary to our hypothesis, no changes

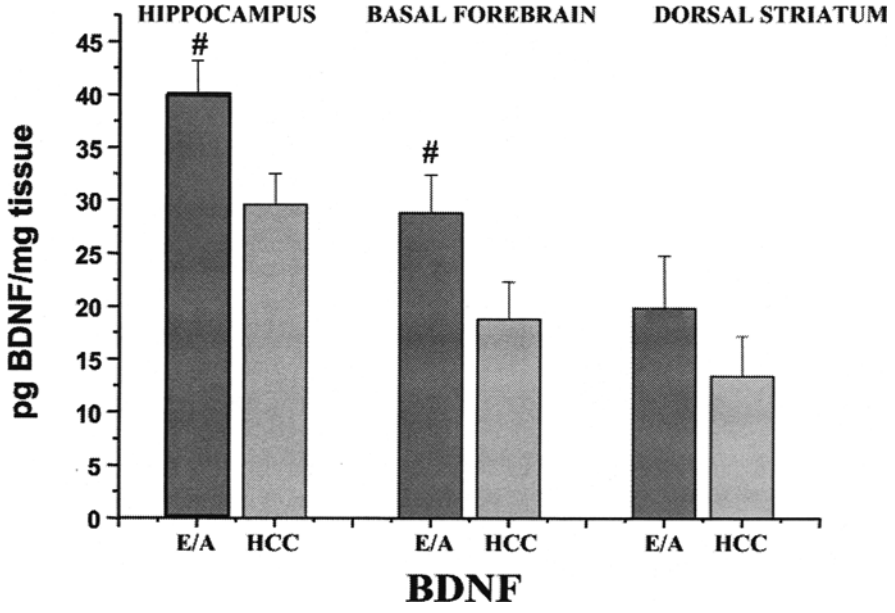

FIG. 2. BDNF values by brain region. Escape/avoidance trained rats (E/A group) had greater ($p < 0.05$, *) BDNF protein than home-cage controls (HCC group) in the hippocampus and showed a trend toward a greater level ($p = 0.06$, #) in the basal forebrain region. BDNF level in the dorsal striatum showed no significant difference ($p > 0.05$) as a function of E/A conditioning.

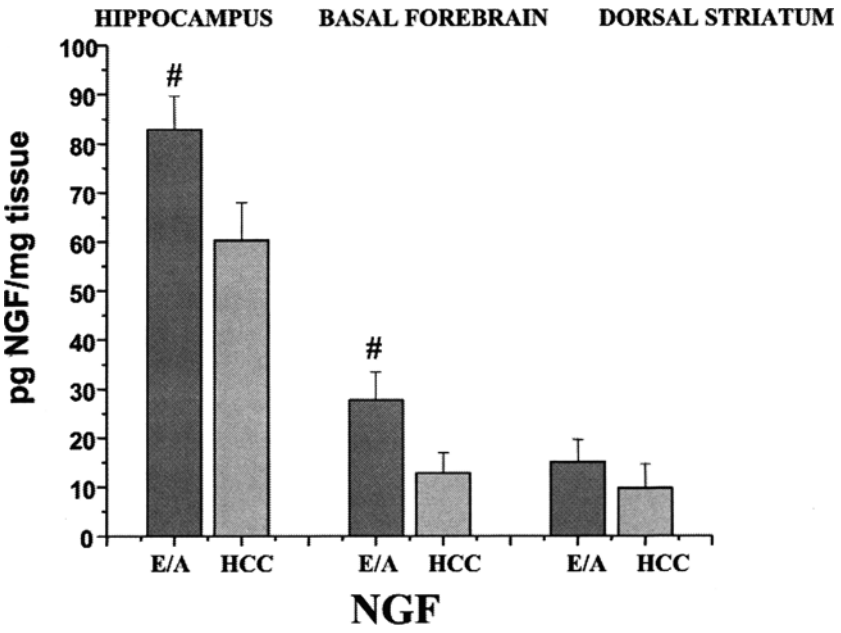

FIG. 3. NGF values by brain region. Escape/avoidance trained rats (E/A group) had greater ($p < 0.05$, *) NGF protein than the home-cage controls (HCC group) in both the hippocampus and basal forebrain regions. NGF level in the dorsal striatum showed no significant difference ($p > 0.05$) as a function of E/A conditioning.

were observed in the dorsal striatum with E/A training, although negative correlations were observed between NGF and measures of avoidance.

Hippocampal neurotrophin levels may be the most sensitive to E/A conditioning, since this is the brain region with the highest level of mRNA and protein for both NGF and BDNF (Wetmore et al., 1990). The abundance of mRNA in the hippocampus means these neurotrophins are locally synthesized. The neurotrophin increase following E/A training is consistent with previous reports in the literature. Although the current procedure is not contextual fear learning per se, it appears obvious that some learning about the chamber must occur. Contextual fear conditioning increases hippocampal BDNF mRNA (Hall, Thomas, & Everitt, 2000) and NGF protein (Woolf et al., 2001).

We have recently found that forced treadmill running, a behavioral task with a greater motor demand and less of a learning component than E/A conditioning, had no effect on BDNF (Kung, Sano, & Albeck, 2003), and decreased hippocampal NGF (Kung, Sano, & Albeck, 2004). In the treadmill task, the rats had to stay on a moving treadmill belt to avoid a mild footshock that was administered if they stopped moving. Perhaps the more demanding learning process required in E/A conditioning stimulated hippocampal BDNF and NGF synthesis, while the learning component in the treadmill task was not strong enough to initiate a neurotrophin increase. Hippocampal NGF protein was significantly decreased in the treadmill rats, opposite to the effect which E/A conditioning induced. Another group has recently reported that two hours of forced walking with no shock present and little to no learning component also reduced hippocampal NGF (Richthofen, Lang, & Hellweg, 2003)

In the basal forebrain, we found an increase in both neurotrophins despite the fact that BDNF (Conner et al., 1997) and NGF (Ceccatelli et al., 1991) mRNA levels are generally low. The positive

correlations between basal forebrain BDNF and fourth hour responses may indicate that BDNF is involved in the motoric aspects of the leverpress response. Consistent with this idea, we have recently found that forced treadmill running caused a significant increase in basal forebrain BDNF (Kung, Sano, & Albeck, 2003) but only in the group required to run at the highest rate.

The dorsal striatum contains no BDNF mRNA (Altar et al., 1997) and has a very low level of NGF mRNA (Bizon, Lauterborn, & Gall, 1999). All of the BDNF and most of the NGF protein in the dorsal striatum must be synthesized in other brain regions and then transported into the striatum (Mufson et al., 1999). Since protein transport takes time, this affects the time-course of protein level changes. Perhaps the dorsal striatum is indeed a site of learning, and the negative correlation between NGF level and avoidance performance indicates that the neurotrophin is being utilized in the striatum, but that sufficient time had not passed in the current experimental design for the used NGF to be replaced. This will be evaluated in a future time course study.

The White and McDonald memory theory would presumably predict synaptic plasticity in the dorsal striatum since the E/A task is an operant paradigm. Although our striatal results found no overall change in BDNF or NGF levels as a function of training, NGF levels negatively correlated with avoidance performance. Although this is clearly preliminary, it appears to support the idea that the striatum may be a locus of learning for this response. It would not be surprising if both the hippocampus and striatum were found to be active during E/A conditioning, since previous studies show that both the hippocampal and striatal memory systems are often simultaneously active in processing information during learning (Packard & Knowlton, 2002).

In summary, we found increases in both NGF and BDNF in both hippocampus and basal forebrain. Future work will attempt to determine the time course of the neurotrophin changes, as well as the relationship of stress to the observed changes.

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