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Extinction-induced "despair" in aged and adult rats: Links to neurotrophins in frontal cortex and hippocampus

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ABSTRACT

In the search for animal models of human geriatric depression, we found that operant extinction of escape from water results in the expression of immobility in different age groups, indicative of behavioral "despair", which was also associated with the resistance-to-extinction (RTE) expressed by these animals. With respect to the neurotrophin hypothesis of depression, nerve-growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) protein levels in frontal cortex (FC) and hippocampus (HP) were examined and related to behavioral immobility and RTE in the water maze in aged and adult Wistar rats. Age-related increases in levels of NGF were found in HP and of NT-3 in FC. Indices of immobility showed relationships in the aged with NGF and, in adults, with BDNF, pointing to a dissociation of neurotrophic involvement in extinction trial-induced "despair" in aged and adult rats. The present results support the hypothesis, that extinction-induced immobility in the water maze reflects a state akin to behavioral despair and point to age-related differences of neurotrophic involvement in depressive-like symptoms. The concept of extinction-induced behavioral "despair" in the aged subsumes several aspects of human geriatric depression, such as co-morbidity of learning impairment and anxiety, and, thus could represent a useful paradigm to examine the neuronal mechanisms underlying depression, especially in aged rodents.

1. Introduction

Geriatric depression is a clinically and neurobiologically heterogeneous disorder that often parallels impairments in cognitive functions (Blazer, 2003; Meltzer et al., 2004) or develops in response to stressful life events, such as social isolation and physical impairment (Prince, Harwood, Blizard, Thomas, & Mann, 1997a, 1997b; for review see: Blazer, 2003). Although clinical trials suggest a good efficacy of antidepressants in treating depression in old age, there is also a high incidence of treatment resistance (Bonner & Howard, 1995; Dew et al., 1997), indicating that some cases of depression in the elderly may be biologically distinct from others. However, studies examining the underlying neuropathology of depressive-like behavior in the aged, especially in rodents, are sparse and new animal models are needed to cover all aspects of the disease.

We have postulated that the withholding of positive and negative reinforcement can lead to depressive-like symptoms. For example, extinction of an escape response from water onto the platform is accompanied by behavioral immobility in aged and adult rats, indicative of behavioral "despair" (Schulz, Huston, Buddenberg, & Topic, 2007; Schulz, Topic, Souza Silva, & Huston, 2004). This immobility behavior shown during extinction is akin to immobility in the forced swimming test, and is alleviated by antidepressant treatment (Schulz, Buddenberg, & Huston, 2007), and thus, held to reflect a depressive state (Porsolt, Le Pichon, & Jalfre, 1977). However, in contrast to the forced swimming test, immobility in the water maze is considered to be the result of withdrawal of reinforcement, previously provided by escape onto a hidden platform. The extinction-induced "despair" model may be also relevant to aged humans who are at a higher risk to experience multiples losses of reinforcers compared to adults. We hypothesized that the degree of resistance-to-extinction should be related to the incidence of "despair". Accordingly, we found that preference for the former platform quadrant (FPQ) during extinction trials was negatively correlated with immobility in

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aged and adult rats (Schulz, Huston et al., 2007), indicating that the higher the resistance-to-extinction (RTE), the lower the incidence of despair. Furthermore, aged rats exhibited higher levels and earlier onsets of immobility over the course of extinction compared to adults (Schulz et al., 2004; Schulz, Huston et al., 2007). The total amount of immobility displayed in aged and adult rats was associated with striatal acetylcholine and dopamine contents in the aged and with noradrenaline contents in adults (Schulz et al., 2004), indicating age-related differences in the underlying neurophysiological mechanisms, and providing further support for the hypothesis of age-related differences in mechanisms underlying depressive-like symptoms.

Several lines of evidence suggest a major role of neurotrophins in the etiology of depression and its treatment, which led to the neurotrophic hypothesis of depression (Duman, 2004), with special emphasis on brain-derived neurotrophic factor (BDNF), nerve-growth factor (NGF) and neurotrophin-3 (NT-3). Acute and chronic stress results in a decrease in levels of neurotrophins in brain regions implicated in the pathophysiology and treatment of depression, such as the hippocampus (HP) and frontal cortex (FC) (for review see: Duman & Monteggia, 2006). Besides their prominent role in the pathophysiology of mood disorders, a dysregulation of neurotrophic factor expression, especially NGF, has also been closely linked to age-related learning and memory deficits as well as neurodegenerative diseases, like Alzheimer's (for review see: Scott & Crutcher, 1994) and Parkinson's disease (for review see: Siegel & Chauhan, 2000).

The present study is a continuation report of results presented in Schulz and Huston et al. (2007). Using a subset of the same animals as before, we here investigated possible age-related differences in neurotrophin protein levels in the HP and FC in animals that were tested in the water maze and submitted to multiple extinction trials. Analysis of the relationships between different neurotrophin protein levels, extinction performance and immobility in aged and adult rats was held to shed more light onto possible age-related differences in neurotrophic mechanisms that may underlie the present model. We hypothesized, that within each age group, the higher the resistance-to-extinction, the less behavioral despair will be shown, in terms of immobility displayed. Furthermore, given the strong relationship between both measures already shown in both age groups (Schulz, Huston et al., 2007), we expected to reveal also relationships of both measures with neurotrophins in both age groups. On the basis of the literature, we expected to find negative associations between indices of "despair" and neurotrophin levels, and thus, more immobility being accompanied by lower neurotrophin levels. In turn, these were expected to be associated with a lower resistance-to-extinction or accelerated extinction performance.

2. Materials and methods

2.1. Animals

Male rats of the outbred Wistar strain obtained from the animal facility of the University of Düsseldorf aged either 3 months ($n = 30$; 409.27 g ± 4.62 *SEM*) or 24 months ($n = 50$; 560.56 g ± 11.74 *SEM*) were maintained under standard laboratory conditions with food and water available *ad libitum* and under a reversed 12:12 light–dark cycle (lights on at 7 pm). Aged animals were housed in standard Makrolon cages with 2–3 animals per cage. Adult animals were housed with five animals per cage. Behavioral testing was conducted during the dark period between 09:00 am and 06:00 pm. All experiments were carried out in accordance with and approved by the German Animal Protection Law (Bezirksregierung Düsseldorf).

Since 11 animals (10 aged and 1 adult) exhibited obvious signs of physical weakness (such as body tumors, eye infection) during the course of experimental testing, their data were excluded from the analysis, resulting in aged: $n = 40$ and adults: $n = 29$. Moreover, due to experimental error or failure to retrieve data post-hoc, individual data points were not available for statistical analysis. All animals were tested every 48 h and underwent the open field, black/white box and elevated-plus maze (refer to Schulz, Huston et al., 2007), followed by the water maze. Approximately 2 weeks after the last test, the brains were taken and 26 animals per age group analyzed for neurotrophin contents. Given the reduced number of subjects available for the present analyses ($n = 26$ per age group), we examined the consistency of the behavioral results with those reported before (Schulz, Huston et al., 2007). Overall, the water maze results were very similar.

2.2. Water maze

The water maze apparatus and experimental design have been described in detail elsewhere (Schulz, Huston et al., 2007). Briefly, a circular 185 cm diameter swimming pool made of black polyethylane was filled 30 cm deep with 20 ± 1 °C water. Rats underwent four trials within one day with the platform cued (a 22-cm high, 0.5-cm diameter metal peg with black and white stripes was fixed onto the platform with a clip and tagged with vanilla aroma). A trial ended when the animal escaped onto the platform or after 1.5 min had elapsed. Two days later the animals were given two training trials per day (one in the morning and one in the afternoon) for 6 days, i.e. the submerged platform was fixed in the center of one quadrant of the maze 1.5 cm below the water surface. Trials were terminated after 2.5 min. This phase was followed by training without a platform present in order to assess extinction of the behavior. The platform was removed and the swimming pattern of the rats was observed during eight extinction trials (one per day) each lasting 3.5 min. Rats were removed from the pool by gently guiding them to the middle of the pool, where they were taken out. They were dried under a red-light heating lamp, before being returned to their home cages. The order of start positions was randomized for each animal and testing day.

The behavioral analysis during acquisition trials for each rat comprised the time to platform (s) and the time spent within the platform quadrant (PQ). The latter was expressed as the percentage of the total trial duration. For extinction, we assessed the time spent in each of the four quadrants. Moreover, the amount of immobility shown by the animals for each extinction trial as a measure of 'learned despair' was recorded, along with the time elapsed for each trial until immobility behavior had set in. Immobility was defined as the lack of motion of the whole body, except that required for balancing to remain afloat. The behavior was not coded if the animal touched the wall of the pool. Furthermore, to differentiate between active and passive (immobility) behavior during the extinction trials, we subtracted the immobility displayed in the former platform quadrant (FPQ) from the amount of time spent in it for each trial, thus, obtaining a measure of 'search time' in the FPQ. In order to assess the resistance-to-extinction, the degree of place preference on day 1 of extinction was taken to be 100% (baseline) and performance on the following days measured relative to day 1. Overall levels as well as slope decreases of place preference (adjusted to a 100% baseline) were computed as measures of resistance-to-extinction. Levels and slopes of immobility could not be adjusted for baseline differences, since most of the scores were zero on day 1 of extinction.