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Cognitive aspects of congenital learned helplessness and its reversal by the monoamine oxidase (MAO)-B inhibitor deprenyl

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ABSTRACT

Cognitive processes are assumed to change with learned helplessness, an animal model of depression, but little is known about such deficits. Here we investigated the role of cognitive and related functions in selectively bred helpless (cLH, $n = 10$), non-helpless (cNLH, $n = 12$) and wild type (WT, $n = 8$) Sprague Dawley rats. The animals were exposed to an open field for 10 min on each of two test days. On the third day, an object exploration paradigm was carried out. The animals were later tested for helplessness. Both cLH and cNLH rats were more active than WTs on the first day in the open field. Over trials, cNLH and WT rats lowered their activity less than cLH rats. This resistance-to-habituation co-varied with a resistance to develop helplessness. In cLH rats, higher 'anxiety' or less time spent in the center of the open field co-varied with severe helplessness. In WTs, a greater reactivity to novel objects and to a spatially relocated object predicted lower levels of helplessness. In cLH rats ($n = 4$–5 per group), chronic treatment with a high dose of the monoamine oxidase (MAO)-B inhibitor deprenyl (10 mg/kg; i.p.), an anti-Parkinson, nootropic and antidepressant drug, attenuated helplessness. Remarkably, helplessness reversal required the experience of repeated test trials, reminiscent of a learning process. Chronic deprenyl (10 mg/kg; i.p.) did not alter locomotion/exploration or 'anxiety' in the open field. In conclusion, helplessness may be related to altered mechanisms of reinforcement learning and working memory, and to abnormalities in MAO-A and/or MAO-B functioning.

1. Introduction

The concept of learned helplessness is used to describe motivational, emotional and cognitive changes that result from uncontrollable stress and resemble symptoms of depression in humans (Overmier & Seligman, 1967; Seligman, 1975). Helplessness is, however, not invariably the consequence of uncontrollable stress (Wieland, Boren, Consroe, & Martin, 1986), suggesting that genetic and/or environmental factors predispose individuals to depression. Genetic animal models have used the technique of selective breeding to generate rat strains that more closely mimic endogenous depression in humans (Brunelli, 2005; Overstreet, 1986; Scott, Cierpial, Kilts, & Weiss, 1996; Will, Aird, & Redei, 2003). Henn and collaborators selectively bred wild type (WT) Sprague Dawley (SD) rats that were either vulnerable or resistant to the effects of inescapable foot-shock (Henn, Edwards, & Muneyyirci, 1993; Henn & Vollmayr, 2005). After more than 50 generations, a congenital learned helpless (cLH) line emerged which exhibits a helpless phenotype even without prior exposure to inescapable stress (IS), whereas the congenital non-helpless (cNLH) line shows resistance to the effects of IS.

Several findings indicate that the cLH strain expresses characteristics consistent with a depressive phenotype. For instance, cLH compared to cNLH rats exhibited anhedonia (a reduction in reward sensitivity) and/or anergia (a reduced persistence to work for reward) under a progressive ratio schedule, hyperactivity in the novel open field, but no spatial learning and memory deficits in the water maze (Vollmayr et al., 2004). In addition, cLH compared to WT controls were found to show aggravated freezing to a conditioned stimulus and a greater resistance-to-extinction of the learned fear response, thus providing a link between a predisposition to 'depression' and an increased propensity to acquire and maintain fear (Shumake, Barrett, & Gonzalez-Lima, 2005). Here we ask about the link between cognition and congenital learned helplessness. Helplessness theory suggests that uncontrollable stress causes subjects to learn that outcomes are not contingent on their responses and alters the ways in which information is processed (Peterson, Maier, & Seligman, 1993). Deficient information processing could affect performance

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in tests of learning and memory. Thus, in this study we chose to examine cLH, cNLH and WT controls in the open field, using a 'hybrid' or multi-domain protocol (Kalueff, LaPorte, Murphy, & Sufka, 2008). This enabled us to assess long-term habituation learning (Cerbone & Sadile, 1994; Leussis & Bolivar, 2006), spatial working memory (Dix & Aggleton, 1999), and also locomotion/exploration (Berlyne, 1966; Montgomery, 1954) and fear (File, 1985). The same animals were then tested for helplessness. To assess whether individual differences in degree of helplessness could be accounted for by variations in the different aspects of open field behavior, we also performed correlation analyses for each group of rats.

Given that cognitive processes are expected to change with helplessness, it is conceivable that drugs which are beneficial to cognitive functioning restore aspects of helplessness or depression. Deprenyl hydrochloride, a monoamine oxidase (MAO)-B inhibitor, specifically inhibits the breakdown of dopamine at low doses and is used to slow the degeneration of neurons in Parkinson's disease (Youdim and Bakhle (2006) for review). It was also shown to improve learning and memory deficits in aged rats without affecting motor performance (Bickford et al., 1997; Brandeis et al., 1991). At high doses, deprenyl was found to reduce immobility in the forced swim test in rats resistant to the effects of tricyclic antidepressants (Kitamura et al., 2008; Shimazu, Minami, Kusumoto, & Yoneda, 2005) and to alleviate treatment-resistant depression in older human patients (Sunderland et al., 1994). Here we tested the effects of different treatment protocols using a high dose of deprenyl on learned helplessness in cLH rats, which, until now, were also considered treatment-resistant (Henn et al., 1993; Vollmayr, Faust, Lewicka, & Henn, 2001).

2. Materials and methods

2.1. Selective breeding

The protocol for selective breeding was developed by Vollmayr and Henn (2001). In short, Skinner boxes equipped with grid floors were employed to deliver altogether 20 min of uncontrollable and unpredictable foot-shock at 0.8 mA. Shock durations and inter-shock-intervals (ITIs) varied between 5 and 15 s. The next day, the animals were tested for learned helplessness by exposure to 15 foot-shocks that lasted 60 s unless turned off earlier by one lever press. The lever was illuminated by a signal light that remained on for the duration of the foot-shock. ITIs were 24 s long. Rats were classified as helpless when they failed to turn off the shock within 20 s on 10 or more trials, but as non-helpless after five or fewer failures. Males and females of each category were bred for over 50 generations at the Central Institute of Mental Health (Mannheim, Germany) to yield a congenital learned helpless (cLH) line and a congenital non-helpless (cNLH) line (Henn & Vollmayr, 2005). The strains were recently transferred to Brookhaven National Laboratory (BNL), where the protocol for selective breeding was maintained, but the set up was modified (Coulbourn Instruments, Allentown, PA) and the current adjusted to 0.4 mA.

2.2. Subjects

Male 3–4 months old cLH ($n = 10$), cNLH ($n = 12$) and WT SD ($n = 8$) rats were tested in the open field and then screened for learned helplessness. The strains were from our breeding colony at BNL and the WTs were purchased from Taconic Farms (Germantown, NY). The animals' weights (g ± SEM) averaged 506 ± 10.6, 464 ± 9.9 and 465 ± 5.3, respectively, a day before the start of the experiments. In the drug studies, 36 adult (3–4 months) and 9 middle-aged (ca. 10 months) male cLH rats were tested for helplessness. Of these 30 adult and all middle-aged rats underwent drug treatment. The remaining animals fell outside the range of our criterion for helplessness ($n = 3$) or were found to be of poor health (external tumors or blood in urine; $n = 3$). An additional 20 adult (3–4 months) male cLH rats were drug-treated and tested in the open field. The animals were housed individually or in groups of 2–3 in standard polycarbonate cages and were maintained on a 12:12 light/dark cycle (lights on at 7.00 am) with free access to food and water. All procedures were approved by BNL's Institutional Animal Care and Use Committee (IACUC) and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*.

2.3. Open field

The open field was a dimly lit (2.15 lx at the center of the field) 60 × 60 cm dark grey mica-coated arena with 40-cm high walls. A video camera (SONY, DCR-SR40) was mounted ca. 2 m above the field; all sessions were videotaped and analyzed with EthoVision software (Noldus, Wageningen, The Netherlands).

The protocol was carried out in accordance with Schulz, Kouri, and Huston (2007). The animals were placed individually into the center of the open field facing the same wall. They were given 10 min on each of two test days, separated by 24 h. On the third day, an object exploration paradigm was carried out. The animals were exposed to the empty open field for 5 min. They were then removed for 1 min and kept in a holding cage while two objects were placed into the field. The objects were blue cups of 10 cm height, 6 cm in diameter on the bottom and 8.5 cm in diameter on the top, with a handle on one side. They were placed upside down across from each other adjoining the walls in their center. The animals were then given 5 min to explore the arena. After that, they were removed once more for 1 min while one of the objects was spatially relocated to one of the remaining walls, diagonal to the stationary object. The animals were placed back into the maze for another 5 min before being returned to the home cage. The experiments took place between 09:00 and 18:00 h. We measured the total distance (cm) moved, the distance moved and time (s) spent in the periphery and center (30 × 30 cm), and the number and duration (s) of rearings in the empty open field. During object exploration the number of contacts with the objects (snout touching the cup) was counted.

2.4. Learned helplessness

Three days after the last open field test, cLH ($n = 10$), cNLH ($n = 12$) and WT SD ($n = 8$) rats underwent helplessness training. Skinner boxes (Coulbourn Instruments, Allentown, PA) equipped with grid floors were employed to deliver 120 uncontrollable foot-shocks at 0.4 mA. Shock durations and ITIs were randomly varied between 5 and 15 s. Up to six animals were run in parallel. The room was equipped with two 40 W lights that provided ca. 2 lx inside the chambers. The next day, the animals were tested for helplessness. In the drug studies, the animals were only tested for helplessness, since cLH rats were used (and express helplessness even without prior exposure to uncontrollable shock). Each box was equipped with a lever and a signal light on top of the lever (providing ca. 320 lx). The lights turned on and off concurrently with the foot-shock. Altogether 15 foot-shocks were delivered that lasted 60 s unless turned off earlier by one lever press. ITIs were 24 s long. Lever presses were automatically recorded by Graphic State software (Coulbourn Instruments, Allentown, PA). The training and test sessions took place between 09:00 and 14:00 h. For each animal, we analyzed the number of lever presses which turned off the foot-shock within 20 s and 60 s of shock onset and evaluated the time required to complete the 15 trials.