Introduction

Seasons differ in the lengths of their photoperiods. Starting July, the hours of daylight progressively shorten until late December when they begin to lengthen again. Some humans are susceptible to these changes and show symptoms known as seasonal affective disorder (SAD), a subtype of major depression. Here, we examined whether antidepressant drugs which regulate monoaminergic function exhibit seasonal effects in an animal model of depression, congenital helplessness.

An abundance of evidence implicates the serotonin (5-HT), norepinephrine (NE) and dopamine (DA) systems in the regulation of seasonal rhythms and the pathophysiology of SAD (3,4). For example, serotonin transporter (SERT) binding was found to vary with season (5,6), and both SERT and dopamine transporter (DAT) binding were reduced in patients with SAD (7,8).

Levels of 5-HT and DA varied with photoperiod in diurnal and nocturnal rodents (9), and following light deprivation markers for apoptosis were increased in monoaminergic neurons, particularly in noradrenergic neurons of the locus coeruleus (10). Here we asked whether antidepressant drugs exhibit seasonal effects given that the underlying neurobiological systems on which they act undergo seasonal changes.
Specifically, we compared the efficacy of the tricyclic antidepressant imipramine and the monoamine oxidase (MAO) inhibitor deprenyl across seasons in a congenital rat model of depression. Model is based on selective breeding of rats that exhibit a helpless phenotype in a behavioral paradigm (11-14). We hypothesized that both imipramine and deprenyl show a seasonal variation with regard to their efficacy in attenuating helplessness. Imipramine binds to the serotonin transporter (SERT) and the norepinephrine transporter (NET) and exerts antidepressant effects at high doses at which the specificity for MAO-B is lost and both MAO-A and MAO-B become inhibited to increase 5-HT, NE and DA (17,18). Thus, any differences in seasonality between imipramine and deprenyl would implicate the DA system specifically, and any differences related to the drugs’ specific mechanisms of action.

Material and Methods

Subjects

We employed naïve male 4-10 months old congenitally helpless (cH, n = 59) rats. The rats were bred at Brookhaven National Laboratory (Upton, NY, USA), where the experiments took place. The animals were derived by selective breeding of wild type Sprague-Dawley rats based on behavioral outcomes in a learned helplessness paradigm (11,12).

The rats were housed in pairs in standard polycarbonate cages and maintained on a 12:12 light:dark cycle (lights on at 7.00 am). They had free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee of Brookhaven National Laboratory and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Selective breeding

The original protocol for selective breeding was developed by Vollmayr and Henn (19), and was slightly altered by Schulz et al. (12). In short, Skinner boxes equipped with grid floors were employed to deliver altogether 20 min of uncontrollable and unpredictable foot-shock. 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.

Rats were classified as helpless when they turned off the foot-shock within 20 s on 5 or fewer trials. Males and females of this category were selectively bred for over 50 generations.

Drugs

Imipramine and deprenyl hydrochloride were purchased from Sigma Aldrich (St. Louis, MO, USA). The drugs were dissolved in 0.9% saline and administered intraperitoneally (i.p.) in a volume of 1 ml/kg at a dose of 10 mg/kg. Control groups received saline.

Procedure

In the present study, cH rats were exposed to helplessness tests only because impairments are present in this strain even without prior exposure to uncontrollable stress (12-14). Test 1 was conducted as a baseline test. The rats were exposed to 15 escapable foot-shocks according to our standard testing protocol. The following day, drug treatment commenced. The animals received imipramine, deprenyl or saline once daily for 14 days. The next day, the animals were re-tested for helplessness (Test 2) using the same protocol as before.

The two days after that, the animals were tested for helplessness again (Test 3 and Test 4, respectively). Drug treatment continued on the re-test days, unless otherwise specified. The injections took place at least 60 min after the behavioral test. Lever presses were automatically recorded by Graphic State software (Coulbourn Instruments, Allentown, PA). For each animal, we analyzed the number of lever presses which turned off the foot-shock within 20 s of shock onset. The experiments were carried out over the course of 2 years in summer and fall (July and November) and in spring (April – May). Rats treated with deprenyl in July (n = 4) and November (n = 5) did not significantly differ from each other in the number of lever presses (all Ps > 0.05), and were therefore pooled (n = 9). Rats treated with deprenyl in spring were also similar, including one batch (n = 5) that was treated for 14 days but not during the re-test days (all Ps > 0.05), and were therefore pooled (n = 15). One batch of animals was treated with imipramine in fall (n = 5) and another in spring (n = 5). cH rats treated with saline in fall (n = 10) and in spring (n = 15) served as controls for the drug-treated groups.

Statistical analysis

All data used for statistical evaluations were checked for normality and equal variance of the distributions, using the Shapiro-Wilk test and Levene-statistic, respectively. Results did not allow for parametric testing to be used in all cases. Accordingly, group comparisons were performed using the Kruskal-Wallis H-test. If statistical differences were revealed (P < 0.05), the Mann-Whitney U-test was carried out for post-hoc comparisons. To compare repeated measures within a group, Friedman tests were applied.
Results

Overall group comparisons revealed that the groups differed from each other in Test 2 (P < 0.001), Test 3 (P < 0.001), and Test 4 (P < 0.001), but not in the baseline test (P = 0.75). Post-hoc comparisons showed that rats injected with imipramine in fall did not differ from rats injected with imipramine in spring (all Ps > 0.05), although imipramine-treated rats performed better than saline-treated controls in fall (Test 2: P = 0.01; Test 3: P = 0.3; Test 4: P = 0.001) and in spring (Test 2: P = 0.02; Test 3: P = 0.06; Test 4: P = 0.008; Fig. 1a). Lever-pressing performance did not significantly increase over the test trials in imipramine-treated rats (fall: P = 0.19; spring: P = 0.26).

By contrast, rats treated with deprenyl in summer-fall turned off the foot-shock more often than rats treated with deprenyl in spring (Test 2: P = 0.005; Test 3: P = 0.06; Test 4: P = 0.03), indicative of a seasonal drug effect (Fig. 1b). Compared with saline, treatment with deprenyl significantly increased the number of lever presses in fall (Test 2: P < 0.001; Test 3: P = 0.005; Test 4: P = 0.001) and in spring (Test 2: P = 0.01; Test 3: P < 0.001; Test 4: P < 0.001). Moreover, deprenyl increased the number of lever presses across trials in fall (P < 0.001) and in spring (P = 0.05).

Rats treated with saline in fall did not differ from rats treated with saline in spring (all Ps > 0.05). In fall, the number of lever presses were similar across trials (P = 0.25), and in spring performance decreased over the trials (P = 0.01).

Figure 1. Seasonal variations in antidepressant drug effects. b) The monoamine oxidase inhibitor deprenyl (10 mg/kg) was more effective in summer-fall than in spring to increase the number of lever presses that turned off aversive foot-shock, indicative of a seasonal drug effect. Compared to saline, deprenyl attenuated helpless behavior in both seasons. *deprenyl vs. saline control; +deprenyl in summer-fall vs. deprenyl in spring.

Discussion

In summary, we found that deprenyl was more effective in summer-fall than in spring to reverse a lever-pressing deficit indicative of helplessness, although deprenyl clearly attenuated the deficit in both seasons. Imipramine was equally effective in improving the lever-pressing deficit in fall and in spring. In both seasons, lever-pressing performance increased over trials indicative of negative reinforcement learning in rats treated with deprenyl, but not in rats treated with imipramine.

Although several studies have reported seasonal variations of static measures, such as levels of 5-HT (20), SERT binding (5,6), and even platelet [3H]imipramine binding (21-23), few studies have observed seasonal variations of dynamic measures, such as physiological and behavioral responses to drugs. In one study, seasonal effects of intravenous L-tryptophan, the precursor of 5-HT, were shown on serum prolactin and plasma tryptophan levels with prolactin entering a trough and tryptophan peaking in the middle of the year (24). Interestingly, Joseph-Vanderpool et al. (25) showed that SAD patients gave higher ratings on the activation/euphoria item of the NIMH self-rating scale when the 5-HT agonist meta-,
chlorophenylpiperazine (m-CPP) was administered in winter as compared to summer. Here, we show for the first time a seasonal drug effect of deprenyl in an animal model of congenital depression. Deprenyl was more effective in treating congenital helplessness in summer-fall than in spring, while it was effective compared to saline in both seasons. By contrast, imipramine was similarly effective in attenuating helplessness in fall and in spring.

Differences in seasonality between the drugs could also directly relate to differences in the drugs’ mechanisms of action. Imipramine binds to the SERT and NET to inhibit the reuptake of 5-HT and NE into the cell, whereas deprenyl binds to MAO to inhibit the breakdown of monoamines. It is surprising that imipramine did not exhibit seasonal variations in its effects on helplessness behavior in light of the many findings that implicate the 5-HT system in circannual rhythms. We cannot exclude that the lack of effect is related to the specific animal model used, drug dose or other method-related differences. Few reports have examined the seasonal rhythmicity of MAO, and they do not conclusively point to the enzyme as key in mediating seasonality.

In humans, platelet MAO was not related with SAD (30). In rats, circadian variations of MAO-A and MAO-B were observed in the brain stem (31), and in mice, components of the circadian clock were found to regulate the expression of MAO-A which, when reduced, led to an increase in striatal DA (32).

Previous work has shown that immobility in the forced swim test, indicative of behavioral despair, was lower in summer-fall and higher in spring (2). The present data show that helplessness was attenuated by deprenyl to a greater extent in summer-fall compared to spring. This could indicate that a) deprenyl is most effective in summer-fall or b) helplessness is less fixed or rigid in summer-fall, and therefore easier to treat. In support of the former, patients with SAD showed a greater responsivity to the 5-HT agonist m-CPP in winter compared to summer (25).

Lastly, we observed that CH rats treated with deprenyl but not imipramine improved their lever-pressing performance across trials, indicative of negative reinforcement learning (12). DA is known to play a prominent role in reinforcement learning, and it has been suggested that reinforcers and DA could promote operant learning through a direct action on memory traces (33-35).

This implies that DA might play a role in the treatment of congenital helplessness, possibly via actions on negative reinforcement learning processes.

**Conclusion**

The present data are the first to demonstrate seasonal effects of deprenyl in the attenuation of helplessness, and most strongly implicate the DA system in these effects. The data support the view that we must take seasonality into account when evaluating the efficacy of antidepressant compounds.

It remains to be seen whether seasonal drug effects can also be found in other animal models of depression, and whether such effects are present with other drug classes, e.g. glutamatergic drugs.

**Conflict of Interest:** The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethical issues:** All Authors declare that originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the authors responsibilities. The study was completed due to defined rules by the Local Ethics Commission guidelines and audits.

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**References**


