Pharmacology Biochemistry and Behavior, Vol. 46, pp 225–230, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Induction of Antidepressive Activity by Monoaminergic Transplants in Rat Neocortex

CARYL E. SORTWELL AND JACQUELINE SAGEN[1]

Department of Anatomy and Cell Biology, University of Illinois at Chicago, 808 S. Wood Street, Chicago, IL 60612

SORTWELL, C. E. AND J. SAGEN. *Induction of antidepressive activity by monoaminergic transplants in rat neocortex.* PHARMACOL BIOCHEM BEHAV 46(1) 225–230, 1993.—To assess the ability of monoaminergic transplants to reduce immobility in the forced swimming test (FST), either adrenal medullary tissue, pineal gland tissue, or equal volumes of sciatic nerve were transplanted into the rat frontal neocortex. In the FST the duration of immobility is thought to indicate the level of antidepressant activity, as immobility times are reliably reduced by antidepressant therapies. Immobility times were reduced in rats with adrenal medullary grafts and pineal grafts to the rat frontal neocortex. In contrast, immobility times were not reduced in control sciatic nerve tissue grafts. Biochemical analysis using HPLC revealed that pineal-grafted neocortex contained higher levels of serotonin (5-HT) and adrenal medullary-grafted neocortex contained higher levels of norepinephrine (NE) than sciatic nerve-grafted or nongrafted controls. Immunocytochemical studies showed that the monoaminergic grafts survived well and continue to produce high levels of monoamines. These results support an important role for neocortical 5-HT and NE transmission in antidepressant activity and suggest that transplants of monoaminergic-containing tissue can reduce biochemical deficits in depression.

Adrenal medulla Pineal Catecholamines Norepinephrine Serotonin Forced swimming test Behavioral despair Depression Neural graft

THE original formulation of the monoamine hypothesis of depression was based on pharmacological evidence that indirectly suggested the involvement of a central deficiency in monoamines in the etiology of affective disorders. Since that time, numerous studies have revealed various abnormalities of the serotonergic and noradrenergic systems in patients with depression (9,11,23,43). Although the literature regarding the exact nature of these abnormalities is inconsistent, overall the monoamine hypothesis remains a helpful framework for the continuing study of the pathophysiology underlying depression (24). Effective antidepressant therapies are proposed to work by correcting the central deficiency in biogenic amines through the reequilibration of monoaminergic systems in the direction of greater efficiency (41,45).

The transplantation of pharmacologically relevant tissues to specific CNS sites is a novel approach toward restoring imbalanced functioning in the CNS. Studies in several laboratories have demonstrated that it is possible to alter behavior by transplanting cells to substitute for lost neurons and neuronal pathways [cf. (14)]. Recently, in our own laboratory it was shown that the transplantation of monoaminergic tissues to the frontal neocortex of rats prevented the development of learned helplessness in a depression model (38). The learned helplessness model is widely considered to be one of the most reliable animal models for depression (46,52). However, because there is some debate over how well the learned helplessness model mirrors depression in humans it is important to use additional animal models.

The forced swimming test (FST), originally named the behavioral despair model when developed by Porsolt in rodents (34), is considered a complementary model that provides further information about the etiology of depression (4). The FST is a popular measure of antidepressant activity that is routinely used for the screening of potential antidepressant drugs. In the FST rats are forced to swim in a confined Plexiglas cylinder and after an initial energetic attempt to escape they assume a readily identifiable immobile posture. On subsequent immersion, the rat assumes the immobile position more rapidly and the duration of immobility is recorded over a 5-min period (32,34). The duration of immobility is significantly reduced in rats treated with a wide variety of antidepressants, including monoamine oxidase inhibitors (MAOIs), tricyclics, and atypical antidepressants (4,16,20,32,34), and chronic treatment potentiates this effect (20,30,31,48). In fact, there is a significant correlation between the potency of antidepressants in the FST swimming test and clinical antidepressants, which gives the FST a level of predictive validity not found in any other model of depression (51,53).

[1] To whom requests for reprints should be addressed.

The aim of this study was to determine whether the transplantation of monoamine-producing tissues into the frontal neocortex could reduce immobility in rats in the FST. The pineal gland was chosen as graft material because it is rich in serotonin (5-HT), containing 5-HT concentrations about 0.5 mM higher than most other brain areas (21), and can be transplanted from nonembryonic donors (26,38). Adrenal medullary tissue served as a source of catecholamines, including norepinephrine (NE). Sciatic nerve tissue served as a nonmonoamine-producing control graft that could be a source of trophic factors. A preliminary account of this study was presented previously (42).

METHOD

Female Sprague–Dawley-derived rats weighing 150 g served as graft recipients for these studies. Two days prior to tissue implantation, FST conditioning and testing was conducted. Each rat was placed in a Plexiglas cylinder (20 cm diameter, 40 cm height) filled with water at room temperature to a height of 15 cm . On the first day of FST testing rats were placed in the water for 15 min for a preliminary conditioning time period and after 24 h were assessed for a 5-min swim period, when immobility scores were recorded, similar to the protocol described by other investigators (32,34). The characteristic immobile posture consisted of a passive upright position where the head of the rat is maintained just slightly above the water level (32,34). Once rats had completed each swim they were removed from the water and placed under a heat lamp until dry.

Graft tissue included rat pineal gland tissue, rat adrenal medullary tissue, or rat sciatic nerve. To prepare the adrenal medullary tissue, adrenal glands were obtained from adult donors and the adrenal cortex was carefully dissected away under a dissecting microscope. Whole pineal glands were also excised from adult donors. As a nonmonoaminergic control, sciatic nerve from adult donors was used. The sheath covering the sciatic nerve was removed prior to transplantation. Approximately two-thirds of an adrenal medulla or one whole pineal gland was transplanted in the monoaminergic transplant groups. Equal volumes of sciatic nerve tissue were transplanted in the control condition. All tissues were cut into small pieces (approximately 0.5 mm^3) in ice-cold Hank's buffer, loaded into a 24-ga injection cannula, and stereotaxically placed into the frontal cortex of anesthetized rats (Nembutal 30 mg/kg, IP; stereotaxic coordinates: A 3.00 mm, L 2.0 mm, H −1.0 mm, incisor bar −2.5 mm from bregma). These frontal coordinates were chosen because this site was shown previously to be a potent cortical site of action for the prevention of learned helplessness by local antidepressant injection (27, 40). These coordinates are also the site where transplantation of monoamine-containing tissue was demonstrated to prevent the development of learned helplessness (38).

Six to 8 weeks following transplantation rats were again conditioned and tested in the manner described above. Prior screening in our laboratory showed that acute treatment with the tricyclic antidepressant imipramine, using a reported effective dosing schedule [30 mg/kg, IP, at 15 min after conditioning swim, 5 h before 5-min test, and 1 h before 5-min test (32)], reduced immobility scores during the 5-min FST swim period, indicating that our behavioral paradigm was sensitive to antidepressant treatment. Animals with either adrenal medullary, pineal, or sciatic nerve transplants were again given 15-min conditioning presessions 24 h prior to 5-min testing. An additional group of control nontransplanted animals previously tested 6 weeks earlier were also reconditioned and retested.

Following the termination of FST behavioral assessment (8 weeks following graft implantation), some rats were sacrificed by decapitation for neurochemical analysis. The neocortical area immediately surrounding the graft was dissected out and weighed. Neocortical tissue samples were extracted in preparation for HPLC analysis using a modification of the method described by Jakubovic et al. (19). Indoleamines were quantitated using reverse-phase high performance liquid chromatography (HPLC) and electrochemical detection over a Waters Resolve C18 column (5 μm particle size, 3.9 × 150 mm) at a flow rate of 1.0 ml/min. Catecholamines were quantitated using reverse-phase HPLC and electrochemical detection over a Waters Resolve C18 μBondapak column (10 μm particle size, 3.9 × 150 mm) at a flow rate of 1.0 ml/min. The serotonin mobile phase contained 0.2 mM octyl sodium sulfate, 0.1 M KH_2PO_4, 0.15 mM EDTA, and 12% methanol at a pH of 4.7. The catecholamine mobile phase contained 0.07 M Na_2HPO_4, 0.2 mM octyl sodium sulfate, 0.1 EDTA, and 2% methanol at a pH of 4.8. The electrochemical detector was set at +0.7 mV vs. an Ag/AgCl reference electrode for indoleamines and at +0.6 mV for catecholamines. All monoamine sample concentrations were calculated and corrected for recovery of authentic monoamines (which was 80–90%) using a Waters Baseline 810 system.

In addition, to assess graft viability, morphological analysis of some graft tissues was performed using immunocytochemistry. To do this, animals were deeply anesthetized with pentobarbital (50 mg/kg) and perfused via the ascending aorta with saline followed by 4.0% paraformaldehyde in a 0.1 M phosphate buffer. CNS tissue containing the grafts was removed and placed in 20% sucrose in phosphate buffer. Twenty-micron sections were cut on a cryostat, preincubated in phosphate-buffered saline containing 1.0% normal goat serum, and exposed to primary antisera to either 5-HT (Incstar, diluted 1 : 500), tyrosine hydroxylase (Incstar, diluted 1 : 500), or preimmune serum. Following incubation overnight at 4°C, sections were washed and exposed to either fluorescein-linked goat anti-rabbit (Cappel, diluted 1 : 100) or rhodamine-linked

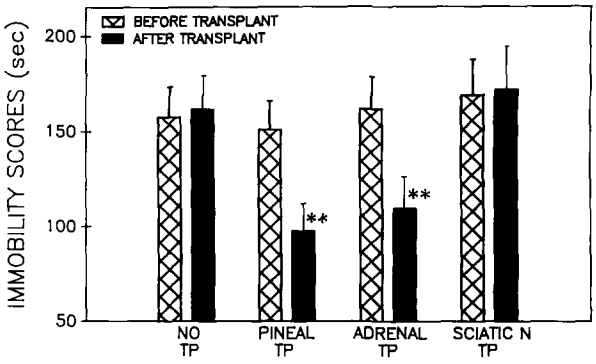

FIG. 1. The effect of frontal cortex transplants on immobility scores in the forced swimming test (FST). Immobility scores were recorded during a 5-min interval on the second day of the FST. Groups of animals included: no transplants (NO TP), pineal transplants (PINEAL TP), adrenal medullary transplants (ADRENAL TP), and sciatic nerve transplants (SCIATIC N. TP). $N = 15$ for each treatment group. Values represent the mean ± SEM for each group before (cross-hatched bars) and 6–8 weeks following transplantation (solid bars). Significant differences are denoted by asterisks ($p < 0.01$).

rabbit anti-mouse (Cappel, diluted 1 : 100) for 1 h at room temperature. Following another wash, sections were mounted on glass slides, coverslipped with Fluoromount, and viewed in a Zeiss Axiophot microscope.

For statistical comparisons between transplant groups, analysis of variance (ANOVA) and the Newman-Keuls test for multiple post hoc comparisons were used. The paired t-test was used to compare immobility scores before and after transplantation.

RESULTS

The average FST immobility scores for the different groups are shown in Fig. 1. The mean immobility scores prior to transplantation were similar in all four groups of animals. Following transplantation, both pineal and adrenal medullary transplants significantly lowered immobility scores [$t(14) = 4.226$ and 3.861, $p < 0.01$, for adrenal and pineal transplant groups, respectively]. This reduction in immobility was similar to that produced by antidepressant treatments in our and other laboratories (32). Both the 5-HT-rich and catecholamine-rich transplants appeared equally effective in reducing immobility. In contrast to the monoamine transplant groups, the mean immobility scores for the sciatic nerve group were not significantly lower than FST immobility scores prior to transplantation, $t(14) = 0.022$, $p > 0.05$. Similarly, immobility scores for nontransplanted animals were not significantly altered during the course of the study, $t(14) = 0.787$, $p > 0.05$.

Figure 2A shows the results from HPLC analysis of the neocortical tissue taken from pineal, sciatic, and nonimplanted animals. 5-HT levels of the pineal-implanted group were significantly higher [overall $F(2, 20) = 13.79$] than both the sciatic-implanted group ($p < 0.01$) and the nonimplanted group ($p < 0.05$). There were no significant differences between the 5-HT levels of the sciatic-implanted group and the nonimplanted group ($p > 0.05$). Figure 2B shows the results from catecholamine analysis of the neocortical tissue taken from adrenal medullary, sciatic, and nonimplanted animals. NE levels of the adrenal medullary-implanted group were significantly higher [overall $F(2, 22) = 5.08$] than both the sciatic and nonimplanted group ($p < 0.05$). There were no significant differences between the NE levels of the sciatic-implanted group and the nonimplanted group ($p > 0.05$). Dopamine (DA) levels of the adrenal medullary-implanted group, although slightly increased, were not significantly different from the sciatic nerve and nonimplanted group [overall $F(2, 22) = 1.05$, $p > 0.05$]. Lastly, epinephrine (EPI) levels of the adrenal medullary-implanted group were also increased [overall $F(2, 22) = 4.11$], but were only significantly higher compared to the nonimplanted group ($p < 0.05$), not the sciatic-implanted group ($p > 0.05$).

Figure 3A shows an adrenal medullary implant in the rat frontal cortex 8 weeks after surgery. This graft has been immunocytochemically stained with a tyrosine hydroxylase antibody. These cells do not stain for 5-HT (not shown). Two distinct clusters of stained cells, possibly in two separate transplant tissue pieces, can be identified. Figure 3B shows a pineal implant to the rat frontal cortex 8 weeks after surgery. The pineal implant is immunocytochemically stained with a 5-HT antibody. The pineal implants are robust, and contain densely packed, 5-HT-containing cells. These cells do not stain with tyrosine hydroxylase antibody (not shown). Occasionally, serotonergic varicosities are observed in regions surrounding the graft not readily visible at this low magnification. The origin

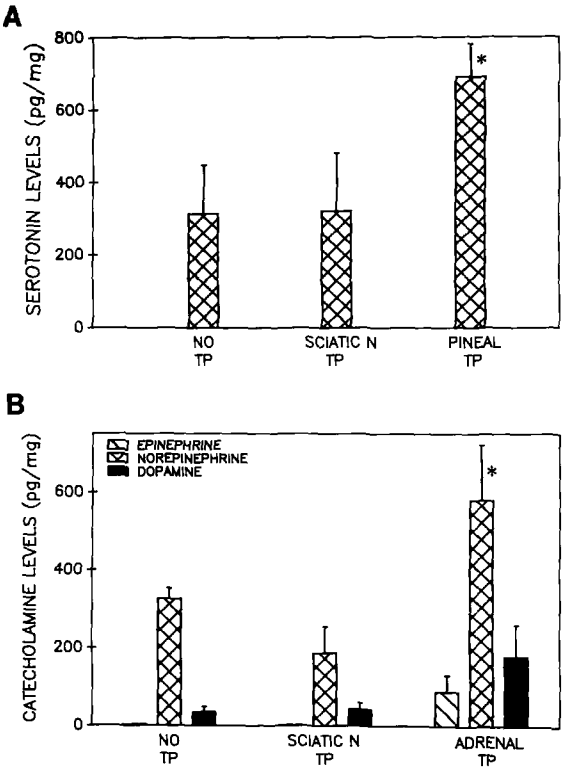

FIG. 2. The effect of frontal cortex transplants on neocortical monoamine levels as assessed biochemically by HPLC analysis. (A) 5-HT levels (pg/mg) for three different treatment groups. Groups of animals included: nontransplanted (NO TP, $n = 7$), sciatic nerve transplants (SCIATIC N., $n = 5$), and pineal transplants (PINEAL TP, $n = 10$). (B) Catecholamine levels (pg/mg) for three different treatment groups. Groups of animals included nontransplanted (NO TP, $n = 8$), sciatic nerve transplants (SCIATIC N. TP, $n = 4$), and adrenal medullary transplants (ADRENAL TP, $n = 12$). EPI levels are represented by single hatch marks, NE levels by double hatch marks, and DA levels by filled bars. Values represent the mean ± SEM for each group. Significant differences are denoted by asterisks ($p < 0.05$).

of these terminals, either from the host or the graft, is not clear, as serotonergic terminals are normally present in this region. Control sciatic nerve implants did not stain for either 5-HT or tyrosine hydroxylase (not shown).

DISCUSSION

The results of this study demonstrat that grafts of monoamine-containing tissues into the frontal neocortex can reduce immobility time in the FST. A reduction of immobility in the FST is thought to reflect an increase in antidepressant activity (4). These findings support previous findings in our laboratory that monoaminergic neural grafts to the same coordinates can prevent learned helplessness, another animal model of depression (38). The probable mechanism of action for the antidepressive effect of the neural grafts is through an increase in local monoamine levels in the frontal neocortex. Consistent with this hypothesis, biochemical studies and immunocytochemical studies show that the grafts survive well and continue to produce high levels of monoamines.

The role of monoaminergic transmission in the reduction of immobility seen in the FST has been the subject of intense

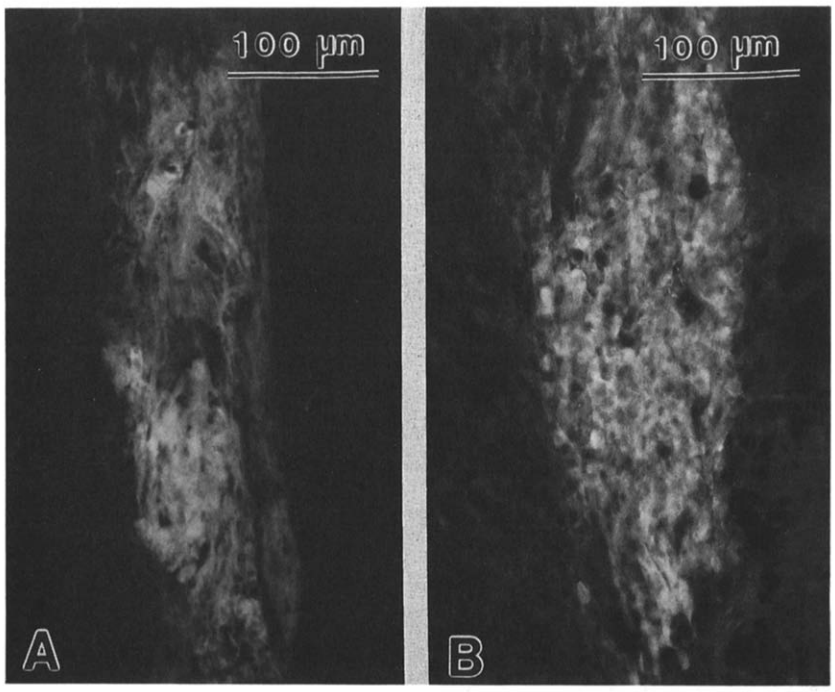

FIG. 3. (A) Adrenal medullary implant in the frontal cortex 8 weeks after transplantation in a rat that had a reduced immobility score in the FST. The cells in the adrenal implant are stained with a tyrosine hydroxylase antibody and a rhodamine-linked secondary antibody. (B) Eight-week-old pineal implant in the frontal cortex of a rat with a reduced immobility score in the FST. The cells in the pineal implant are stained with a 5-HT antibody and a fluorescein-linked secondary antibody.

investigation. Evidence from other laboratories suggests the involvement of both noradrenergic (28,29,50) and serotonergic (5) systems as well as a possible role for DA (6). Pharmacological agents that affect central 5-HT and catecholamine activity have been demonstrated to reduce immobility as well (8, 33), although there is a lack of agreement as to what specific monoaminergic receptors may be responsible for the antidepressant effects (7,13). In addition, chronic treatment with the tricyclic desipramine was found to increase the noradrenergic activity as measured by MHPG-SO_4 levels in only a few specific rat brain regions, the frontal cortex being one of them (25). Although the effect of direct monoamine injections into the frontal cortex on the FST has not been previously reported, preliminary findings in our laboratory also indicate that local injections of 5-HT or NE, but not saline, to the frontal neocortex of rats also reduce immobility time in the FST.

The biochemical assay results of the present study implicate increases in either 5-HT or NE, but not DA, in the antidepressive effects of the pineal or adrenal medullary grafts, respectively, since cortical dopamine levels were not increased. The failure of adrenal medullary transplants to increase cortical dopamine is interesting in light of its choice as donor material for replacement of lost DA neurons in Parkinson's disease models [cf. (14)]. Other studies in our laboratory have similarly failed to reveal increased DA levels following adrenal medullary transplantation in CNS pain modulatory regions (37).

In the present study, donor tissues were chosen for their high levels of monoamines. Per gram, the pineal contains as much as 50 times as much 5-HT as the entire brain (10). Although pineal 5-HT is present as a precursor to melatonin, 5-HT itself is also released from the pineal gland (2,15,39,49). Furthermore, denervation of the pineal gland may reduce the activity of *N*-acetyltransferase, the enzyme converting serotonin to melatonin, thereby increasing 5-HT levels (18,21, 35,44). However, a role for melatonin release from transplanted pineal cells cannot be eliminated in the present study, as melatonin has also been implicated in the etiology of depression (1).

Similarly, catecholamines are not the only substances released from the adrenal medulla. It has been shown that high levels of diffusible opioid peptides are also released from transplanted chromaffin cells (36). Therefore, it is possible that the release of opioid peptides may play a role in the decreased immobility as well. This possibility deserves investigation, as other studies have revealed that the blockade of opioid receptors reduces the antidepressant effect of tricyclic antidepressants on the FST (12).

Recent findings in other laboratories have suggested that some of the beneficial effects of neural transplants are due to the release of trophic factors rather than the monoamines themselves (3,22). To address this, sciatic nerve was transplanted as a control, since it is generally accepted that the Schwann cells of sciatic nerve produce nerve growth factor (NGF), with levels of NGF increasing dramatically after sciatic nerve transection (17,47). The results that sciatic nerve transplants did not elevate monoamine levels or reduce immobility scores suggest that the grafts are not producing their effects via trophic factor stimulation of the host CNS.

Another possible explanation for the reduction in immobility by monoaminergic transplants is an effect on locomotor

activity. Since drugs enhancing motor activity may give a false positive in the FST, open field activity is sometimes also measured (32). A pilot study in our laboratory revealed no differences in locomotor activity between transplant groups. This suggests that locomotor effects are probably not the mechanism for decreased immobility by monoaminergic transplants. Nevertheless, until this question is investigated more extensively this possibility cannot be completely eliminated.

In summary, results of this study suggest that monoaminergic neural transplants can provide a local source of monoamines for antidepressive action. The heterogeneity of human affective disorders does not lend itself easily to mirroring by only one animal model of depression. The ability of monoaminergic neural grafts to alleviate depression in two different animal models is therefore an important and promising finding and adds credence to the possibility of monoaminergic neural transplants providing a new approach to the treatment of clinical depression.

ACKNOWLEDGEMENTS

We are grateful for the technical assistance in the biochemical analysis of HPLC samples from Mrs. Mary Lavallee and Mr. John Jenco. We appreciate the financial support from Dr. John M. Davis, Illinois State Psychiatric Institute.

REFERENCES

1. Beck-Friis, J.; Wetterberg, L. Melatonin and the pineal gland in depressive disorders. In: Halbreich, U., ed. Hormones and depression. New York: Raven; 1987:195–206.
2. Benson, B.; Reynolds, W. D. Norepinephrine stimulated release of pineal serotonin may be mediated by alpha adrenergic receptors. Soc. Neurosci. Abstr. 14:25; 1988.
3. Bohn, M. C.; Cupit, C.; Marciano, F.; Gash, D. M. Adrenal medullary grafts enhance recovery of striatal dopaminergic fibers. Science 237:913–916; 1987.
4. Borsini, F.; Meli, A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berlin) 94:147–160; 1988.
5. Cervo, L.; Grignaschi, G.; Nowakowska, E.; Samanin, R. 1-(3-Trifluoromethylphenyl)piperazine (TFMPP) in the ventral tegmental area reduces the effect of desipramine in the forced swimming test of rats: Possible role of serotonin receptors. Eur. J. Pharmacol. 171:119–125; 1989.
6. Cervo, L.; Grignaschi, G.; Samanin, R. The role of mesolimbic dopaminergic system in the desipramine effect in the forced swimming test. Eur. J. Pharmacol. 178:129–133; 1990.
7. Cervo, L.; Grignaschi, G.; Samanin, R. α_2-adrenoreceptor blockade prevents the effect of desipramine in the forced swimming test. Eur. J. Pharmacol. 175:301–307; 1990.
8. Cervo, L.; Samanin, R. Effect of chronic treatment with 8-OH-DPAT in the forced swimming test requires the integrity of presynaptic serotonergic mechanisms. Psychpharmacology (Berlin) 103:524–528; 1991.
9. Charney, D. S.; Menkes, D. B.; Heninger, G. R. Receptor sensitivity and the mechanism of action of antidepressant treatment: Implications for the etiology and therapy of depression. Arch. Gen. Psychiatry 38:1160–1180; 1981.
10. Cooper, J. R.; Bloom, F. E.; Roth, R. H. The biochemical basis of neuropharmacology. New York: Oxford University Press; 1991:346.
11. Davis, J. M.; Bresnahan, D. Psychopharmacology in clinical psychiatry. In: Psychiatry update: American psychiatric association annual review, vol. 6. Washington, DC: American Psychiatric Press; 1987.
12. Devoize, J. L.; Rigal, F.; Eschalier, A.; Trolese, J. F.; Renoux, M. Influence of naloxone on antidepressant drug effects in the forced swimming test in mice. Psychopharmacology (Berlin) 84: 71–75; 1984.
13. Duncan, G. E.; Breese, G. R.; Criswell, H.; Stumpf, W. E.; Mueller, R. A.; Covey, J. B. Effects of antidepressant drugs injected into the amygdala on behavioral responses of rats in the forced swim test. J. Pharmacol. Exp. Ther. 238:758–762; 1986.
14. Dunnett, S. B.; Richards, S. J., eds. Progress in brain research, vol. 82. Neural transplantation: From molecular basis to clinical applications. New York: Elsevier; 1990.
15. Fuller, R. W.; Perry, K. W. Increase of pineal noradrenaline concentration in rats by desipramine but not fluoxetine: Implications concerning the specificity of these uptake inhibitors. J. Pharm. Pharmacol. 29:710–711; 1977.
16. Gorka, Z.; Wojtasik, E. The effect of antidepressants on behavioral despair in rats. Pol. J. Pharmacol. Pharm. 32:463–468; 1980.
17. Heumann, R.; Korsching, S.; Bandtlow, C.; Thoenen, H. Changes of nerve growth factor synthesis in non-neuronal cells in response to sciatic nerve transection. J. Cell. Biol. 104:1623–1631; 1987.
18. Howell, H. E.; Morgan, P. J. β-adrenergic stimulation increases cAMP and melatonin production in ovine pinealocyte cultures. J. Pineal Res. 10:122–129; 1991.
19. Jakubovic, A.; Fu, D.; Fibiger, H. C. Factors affecting the stability and separation of biogenic amines and their metabolites: Simultaneous measurement by HPLC with electrochemical detection. J. Pharmacol. Methods 17:1–14; 1987.
20. Kitada, Y.; Miyauchi, T.; Satoh, A.; Satoh, S. Effects of antidepressants in the rat forced swimming test. Eur. J. Pharmacol. 72: 145–152; 1981.
21. Klein, D. C.; Auerbach, D. A.; Namboodiri, M. A. A.; Wheeler, G. H. T. Indole metabolism in the mammalian pineal gland. In: Reiter, R. J., ed. The pineal gland: Anatomy and biochemistry, vol. 1. Boca Raton: CRC Press; 1981:199–227.
22. Kordower, J. H.; Felten, D. L.; Gash, D. M. Dopaminergic transplants for Parkinson's disease. In: Olanow, C. W.; Lieberman, A., eds. Scientific basis for therapy inParkinson's disease. London: Parthenon Press; 1992:175–224.
23. Korpi, E. R.; Kleinman, J. E.; Goodman, S. I. Serotonin and 5-HIAA in brains of suicide victims: Comparison in chronic schizophrenic patients with suicide as cause of death. Arch. Gen. Psychiatry 43:594–600; 1986.
24. Martin, M. B.; Owen, C. M.; Morihisa, J. M. An overview of neurotransmitters and neuroreceptors. In: Hales, R. E.; Yudofsky, S. C., eds. The American psychiatric press textbook of neuropsychiatry. Washington, DC: American Psychiatric Press; 1987:55–85.
25. Miyauchi, T.; Kitada, Y.; Satoh, S. Effects of acutely and chronically administered antidepressants on the brain regional 3-methoxy-4-hydroxyphenylethyleneglycol sulfate in the forced swimming rat. Life Sci. 29:1921–1928; 1981.
26. Nonaka, T.; Araki, M.; Kimura, H.; Nagatsu, I.; Satoh, F.; Masuzawa, T. Transplantation of the rat pineal organ to the brain: Pinealocyte differentiation and innervation. Cell Tissue Res. 260:273–278; 1990.
27. Petty, F.; Sherman, A. D. Regional aspects of the prevention of learned helplessness by desipramine. Life Sci. 26:1447–1452; 1980.
28. Plaznik, A. P.; Danysz, W.; Kostowski, W. Mesolimbic noradrenaline but not dopamine is responsible for organization of rat behavior in the forced swim test and an anti-immobilizing effect of desipramine. Pol. J. Pharmacol. 37:347–357; 1985.
29. Plaznik, A. P.; Danysz, W.; Kostowski, W. A stimulatory effect of intraacumbens injections of noradrenaline on the behavior of rats in the forced swim test. Psychopharmacology (Berlin) 87: 119–123; 1987.
30. Poncelet, M.; Gaudel, G.; Danti, S.; Soubrie, P.; Simon, P.

Acute vs. repeated administration of desipramine in rats and mice: Relationships between brain concentrations and reduction of immobility in the swimming test. Psychopharmacology (Berlin) 90:139–141; 1986.
31. Porsolt, R. D. Behavioral despair. In: Enna, S. J.; Malick, J. B.; Richelson, E., eds. Antidepressants: Neurochemical, behavioral, and clinical perspectives. New York: Raven Press; 1981: 121–139.
32. Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. Behavioral despair in rats: A new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47:379–391; 1978.
33. Porsolt, R. D.; Bertin, A.; Blavet, N.; Deniel, M.; Jalfre, M. Immobility induced by forced swimming rats: Effects of agents which modify central catecholamine and serotonin activity. Eur. J. Pharmacol. 57:201–210; 1979.
34. Porsolt, R. D.; Le Pichon, M.; Jalfre, M. Depression: A new model sensitive to antidepressant treatments. Nature 266:730–732; 1977.
35. Rowe, V.; Parr, J. Developmental changes in the stimulation of serotonin *N*-acetyltransferase activity and melatonin synthesis in the rat pineal monolayer culture. J. Pharmacol. Exp. Ther. 218: 97–102; 1981.
36. Sagen, J.; Kemmler, J. E. Increased levels of met-enkephalin-like immunoreactivity in the spinal cord CSF of rats with adrenal medullary transplants. Brain Res. 502:1–10; 1989.
37. Sagen, J.; Kemmler, J. E.; Wang, H. Adrenal medullary transplants increase spinal cord cerebrospinal fluid catecholamine levels and reduce pain sensitivity. J. Neurochem. 56:623–627; 1991.
38. Sagen, J.; Sortwell, C. E.; Pappas, G. D. Monoaminergic neural transplants prevent learned helplessness in a rat depression model. Biol. Psychiatry 28:1037–1048; 1990.
39. Shein, H. M.; Wurtman, R. J.; Axelrod, J. Synthesis of serotonin by pineal glands of the rat in organ culture. Nature 213:730; 1967.
40. Sherman, A. D.; Petty, F. Neurochemical basis of the action of antidepressants on learned helplessness. Behav. Neural Biol. 30: 119–134; 1980.
41. Siever, L. J.; Davis, K. L. Overview; Toward a dysregulation hypothesis of depression. Am. J. Psychiatry 142:1017–1031; 1985.
42. Sortwell, C. E.; Sagen, J. Monoaminergic neural transplants to the neocortex reduce immobility time in rats during behavioral despair testing. Soc. Neurosci. Abstr. 16:654; 1990.
43. Stanley, M.; Mann, J. Increased serotonin$_2$ binding sites in the frontal cortex of suicide victims. Lancet 1:214–216; 1983.
44. Steinlechner, S.; King, T. S.; Champney, T. H.; Richardson, B. A.; Reiter, R. J. Pharmacological studies of the regulation of *N*-acetyltransferase activity and melatonin content of the pineal gland of the Syrian hamster. J. Pineal Res. 2:109–119; 1985.
45. Syvalanti, E. Monoaminergic mechanisms in affective disorders. Med. Biol. 65:85–96; 1987.
46. Telner, J. I.; Singhal, R. L. Psychiatric progress; The learned helplessness model of depression. J. Psychiatr. Res. 18:207–215; 1984.
47. Thoenen, H.; Bandtlow, C.; Heumann, R. The physiological function of nerve growth factor in the central nervous system: Comparison with the periphery. Rev. Physiol. Biochem. Pharmacol. 109:145–178; 1987.
48. Trzeciak, H. I.; Janas, P.; Herman, Z. S. Effects of chronic oral administration of imipramine in the rat forced swimming test. Pol. J. Pharmacol. 38:179–183; 1986.
49. Walker, R. F.; Sparks, D. L.; Slevin, J.; Rush, M. E. Temporal effects of norepinephrine on pineal serotonin in vitro. J. Pineal. Res. 3:33–40; 1986.
50. Weiss, J. M.; Simson, P. G.; Hoffman, L. J.; Ambrose, M. J.; Cooper, S.; Webster, A. Infusion of adrenergic receptor agonists and antagonists into the locus coeruleus and ventricular system of the brain. Neuropharmacology 25:367–384; 1986.
51. Willner, P. The validity of animal models of depression. Psychopharmacology 83:1–16; 1984.
52. Willner, P. Validation criteria for animal models of human mental disorders: Learned helplessness as a paradigm case. Prog. Neuropsychopharmacol. Biol. Psychiatry 10:677–690; 1986.
53. Willner, P. Animal models as simulations of depression. Trends Pharmacol. Sci. 12:131–136; 1991.