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Cerebral Correlates of Depressed Behavior in Rats, Visualized Using ^{14}C-2-Deoxyglucose Autoradiography

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^{14}C-2-Deoxyglucose (2DG) was used to investigate changes in the rate of cerebral metabolism in 3 rat models of depressed behavior. The models had already been established in the literature and were induced by injections of α-methyl-para-tyrosine, withdrawal from chronic amphetamine, or stress. We verified that exploratory behaviors were depressed in each model and that an antidepressant drug, tranylcypromine, prevented the depressed behavior in each model. 2DG studies revealed that the rate of regional glucose metabolism was elevated bilaterally in the lateral habenula of each of the 3 models. Regional metabolic rates were reduced in each model in the dorsal medial prefrontal cortex, anterior ventral nucleus of the thalamus, and inferior colliculus. Forebrain global metabolic rates were also reduced in each of the models. Tranylcypromine prevented the elevated rate of lateral habenula metabolism seen in each of the models alone but did not significantly affect the rates of global metabolism. Our findings of identical metabolic changes in each of the models indicate that these changes are not idiosyncratic to a particular model; rather, they correlate with a generalizable state of depressed exploratory behavior in rats.

Although depression in humans is extremely complex, a number of animal preparations have been described in the literature as modeling some component behaviors of the human depressed state. Similar to humans, animal models have exhibited various reductions in behavior or alterations in physiological states. For example, reduced motor activity has been reported in a number of stress models (McKinney and Bunney, 1969; Porsolt et al., 1978; Hatotani et al., 1979; Seligman et al., 1979; Sherman et al., 1979; Katz et al., 1981a; Crawley, 1984) and drug models (Rech et al., 1966; Lynch and Leonard, 1978) of depressed behavior. Reduced social contact has also been described in stress (Kaufman and Rosenblum, 1967; McKinney and Bunney, 1969; Crawley, 1984) and drug models (Redmond et al., 1971). Likewise, decreased learning (Seligman et al., 1979; Sherman et al., 1979; Leonard and Tuite, 1981) and reduced reward behaviors (Cassens et al., 1981; Katz, 1982) have been reported in various models. Finally, models of depressed behavior have demonstrated altered hormone levels (Katz et al., 1981a; Leonard and Tuite, 1981) and abnormal circadian cycles (Hatotani et al., 1979; Cahill and Ehret, 1982). These behavioral and physiological alterations in the animal models have also been reversed by antidepressant drugs.

The advantage of these animal studies is that they used invasive techniques, unlike human research, to examine anatomical structures that were implicated in mediating depressed behaviors. For example, lesions of the fornix or the septum were found to prevent induction of a learned helplessness model of depressed behavior (Beagley and Beagley, 1978; Leshner and Segal, 1979). Also, injections of GABA or norepinephrine into the hippocampus (Sherman and Petty, 1980) prevented learned helplessness. Likewise, catecholamines injected into the accumbens nucleus prevented depressed behavior induced by a forced swim model (Plaznik et al., 1985). Finally, the number of β-adrenergic receptors was increased and imipramine receptors were decreased respectively in the hippocampus and prefrontal neocortex of rats in the learned helplessness model (Johnson et al., 1982).

While these animal studies represented a desirable beginning in understanding brain mechanisms that may mediate components of depressed behavior, several aspects of their experimental designs were problematic. For example, the receptor binding study (Johnson et al., 1982) was the only one in which all anatomical structures in the brain were assessed for changes that correlated with the reduced behavior. The other experimental designs, in which a few brain structures were preselected for experimental manipulation, obscured the fact that additional brain structures may have been equal or more important in mediating the reduced behaviors. Second, each study used only one model of depressed behavior. Under such circumstances, it is not possible to determine whether the demonstration of a brain structure's involvement in a behavior is generalizable to other models of similar depressed behaviors. When studying such models, the goal should be to find brain structures that mediate the generalized state of depressed behavior, rather than correlating with some idiosyncratic aspect of a particular model. Third, while lesions, intracerebral injections, and receptor binding studies are feasible in animals, such data are difficult to extrapolate to humans, and comparative data are therefore scarce.

In order to increase the understanding of what brain structures may mediate depressed behaviors, we sought to solve the problems limiting interpretation of the previous animal studies. The ^{14}C-2-deoxyglucose (2DG) autoradiographic technique (Sokoloff et al., 1977) was used to measure glucose metabolism in the brains of rats given various models of depressed behavior. This radiographic technique allowed us to survey the entire brain for

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functional changes in specific anatomical structures that may have been involved in the production or modification of a particular behavior. Furthermore, 2DG studies have the same tracer kinetic basis as human studies using 18-fluorodeoxyglucose and positron emission tomography. Thus, results from our animal studies are directly comparable to human data, while also allowing for invasive manipulations that could not be attempted in humans. Finally, we studied 3 different rat models of depressed behavior, and only results that were the same in all 3 models were considered as true correlates of the depressed behaviors. This criterion precluded results that were idiosyncratic to one model and, instead, favored results that were applicable to a generalized state of depressed behavior.

The behaviors we studied were involved with exploration of a novel environment. The models were respectively induced by injections of the tyrosine-hydroxylase inhibitor, α-methyl-para-tyrosine (Rech et al., 1966), withdrawal from chronic amphetamine (Seltzer and Tonge, 1975; Cassens et al., 1981) or chronic stress (Katz et al., 1981a). Some of our behavioral results, showing reductions in exploration, corroborated those of previous studies in these same models. However, we also found changes in the rates of forebrain global and regional cerebral glucose metabolism that correlated with reduced exploratory behavior. Furthermore, many of these changes in metabolism and behavior were prevented by the administration of the antidepressant, tranylcypromine. Our results are particularly interesting in that they parallel results by our group of similar changes in supratentorial global and regional glucose metabolism in bipolar depressed patients (Phelps et al., 1984; Baxter et al., 1985).

Materials and Methods

Subjects. A total of 207 adult, male Sprague-Dawley rats (250–350 gm) were studied in these experiments. One hundred and thirty-three rats were used for behavioral studies, and 74 rats were used for 2DG studies. In order to correlate changes in behavior accurately with brain glucose metabolism, the times for behavioral testing and for 2DG administration following the end of model induction had to be identical. Thus, separate groups of animals were used for these 2 procedures. Animals were individually housed and maintained on a normal 12-hr light cycle with food and water available at all times except during certain phases of the stress-induction procedure.

Induction of the 3 models of depressed behavior. α-Methyl-para-tyrosine (AMPT) was administered as an aqueous suspension (50 mg/kg, IP) at 11:00 p.m., 3:00 a.m., and 7:00 a.m. during the same 12 hr period (Rech et al., 1966). Either behavioral testing or 2DG infusion began 5 hr after the last AMPT injection. This time interval was shown to correlate with significant depletions in brain norepinephrine and dopamine concentrations (Rech et al., 1966).

Amphetamine base was extracted from amphetamine sulfate using sodium hydroxide, ether, and a drying agent, magnesium sulfate. After separation, the remaining ether was blown off in an argon atmosphere to prevent oxidation of the amphetamine base. The base was then mixed with a nonoxidizing vehicle, polyethylene glycol (PEG 300), and the mixture was administered via pellets implanted subcutaneously in the backs of the rats (Huberman et al., 1977) under halothane anesthesia. The pellets were made of a 25 mm length of 7.9 mm diameter Silastic medical grade tubing (Dow Corning) with 20 mm inserts of polyethylene tubing to retard the diffusion rate of the amphetamine (PEG cannot diffuse across the wall of the pellet). The ends of the pellet were sealed with Silastic polymer (382 Medical Grade Elastomer, Dow Corning), similar to the procedure of Huberman et al. (1977). Each pellet initially contained the equivalent of 94.6 mg/kg (28 mg in a 300 gm rat) of the base in polyethylene glycol. The pellet was removed (again under halothane anesthesia) 4 d after surgical implantation, and 2DG or behavioral testing was begun 20 hr later during a period of withdrawal from amphetamine (Amp WD). Although brain concentrations of norepinephrine and dopamine were not measured during this withdrawal period, these catecholamines were reported to be reduced 2–5 d after pellet implantation (Ellison et al., 1978).

Induction of chronic stress consisted of 20 d of the following stressors, delivered in a semirandom fashion according to Katz et al. (1981a): exposure to 60 min of foot shock varying from 1 to 8 sec in duration at 1 mA, 40 hr of food deprivation, cold swim at 7°C for 5 min, 40 hr of water deprivation, 5 min exposure to heat at 40°C, 30 min of shaking in a Fisher rotator (200 rotations/min), reversal of the day/night cycle, and changing of cage mates. On day 21, rats were exposed to bright lights and 88 dB of white noise for 1 hr; they were then immediately injected with 2DG or tested behaviorally, similar to the behavior testing reported by Katz et al. (1981a).

Administration of the antidepressant drug. Tranylcypromine is a monoamine oxidase (MAO) inhibitor with a rapid onset of action (within 48 hr; *Physicians' Desk Reference,* 1984). It was administered in order to prevent the alterations of metabolism that correlated with reduced exploratory behavior. The drug doses and injection protocols were copied from previous studies of tranylcypromine's effects on the AMPT and stress models. In the AMPT model, the drug was injected in a single dose, 10 mg/kg IP 17 hr prior to AMPT administration (30 hr prior to 2DG or behavioral testing), similar to Moore and Rech (1967). In the amphetamine withdrawal model, tranylcypromine was also injected once, 10 mg/kg IP, immediately following removal of the amphetamine-containing pellet (20 hr prior to testing). In the stress model, tranylcypromine was administered once a day, 10 mg/kg IP, concurrent with stress induction, for 3 weeks prior to 2DG or behavioral testing (according to Katz et al., 1981b). Control rats were divided into 2 groups and given 10 mg/kg tranylcypromine, IP, either as a single injection or for 3 weeks. The use of different tranylcypromine administration protocols was derived empirically. Initial efforts to inject all animals for 3 weeks prior to and during induction of the models (the protocol that is more effective clinically) caused most of the amphetamine withdrawal rats to die. The lethality of this combination is not surprising in light of the contradictions, described in the *Physicians' Desk Reference,* for the simultaneous use of MAO inhibitors and sympathomimetics.

Behavioral experiments. These experiments were performed prior to the 2DG studies in order to verify reports in the literature of reduced exploratory behavior in each of the 3 models of depressed behavior. Two components of exploratory behavior were tested, locomotion and rearing in an open-field apparatus, where this environment was novel to the rat. Testing occurred at the intervals following AMPT administration, removal of the amphetamine pellet, or presentation of the final stressor as described above. All tests were performed between 10:00 a.m. and 1:00 p.m. so as to hold the phase of the diurnal cycle constant across groups. Behavior was measured by placing rats in a circular cage (1 m diameter) with squares (8 × 8 inches) marked on the floor and counting both the number of squares crossed and the number of rears performed per minute during a 6 min observation period. In each of the 3 rat models, behavior was tested in the presence and absence of tranylcypromine. The squares and rearing data were each analyzed using one-way analyses of variance for the various model, model + antidepressant, antidepressant, and control groups. *Post hoc* Newman-Keuls tests (at the $p < 0.05$ level) were then used to analyze each of the experimental groups separately.

2DG studies. The animals were prepared for 2DG administration by having polyethylene catheters inserted into one femoral artery and vein, respectively, under halothane anesthesia. At the end of this surgery, all wound margins were infiltrated with xylocaine, and rats were restrained from the abdomen caudally by a loose-fitting plaster cast. Three to four hours were allowed for recovery from surgery before administering 2DG. The infusion of 2DG occurred at the times, following administration of AMPT, withdrawal from amphetamine, or presentation of the final stressor, described above. In all animals, 2DG was administered between 12:00 and 1:00 p.m. 2DG, 14–20 μCi/100 gm body weight in 0.5 ml normal saline (specific activity, 50–55 mCi/mmol; American Radiolabelled Chemicals) was injected intravenously over 30 sec. Timed arterial blood samples were collected during the subsequent 45 min. The blood samples were centrifuged immediately in a Beckman microfuge, and the plasma was separated and stored on ice until further analysis. The temperature of the animals was maintained between 37 and 38°C throughout the experiment. At the end of the 45 min experiment, animals were injected with 0.15 cc sodium pentobarbital (Nembutal) IV to produce anesthesia and then decapitated; the brains were rapidly removed, frozen in dry ice, and later sectioned on a cryostat

into 20 μm sections, which were thawed onto coverslips and dried on a warming tray. Together with calibrated poly-^{14}C-methyl methacrylate standards (Amersham), the sections were exposed against X-ray film (Kodak NMC-1) for 7–14 d; the film was then developed in an automatic developing machine (Kodak X-OMAT, model SP). The arterial plasma samples were assayed for 2DG concentration by liquid scintillation counting, and for glucose concentration using a Beckman glucose analyzer.

The autoradiograms were, initially, visually inspected to determine which brain areas showed changes in optical densities. Next, optical densities were measured bilaterally in 23 brain regions, as well as in 2 midline regions. Measurements were made using a densitometer with a 0.25 mm aperture (Sargent Welch) or a computer-based automated image analyzer (Zeiss IBAS II Image Analyzer). Forebrain global densities of all brain structures anterior to the midbrain and dorsal to the hypothalamus (except the medial geniculate nuclei, whose densities changed with the level of noise in the laboratory) were also measured, using the IBAS image analyzer. Knowing the plasma glucose concentrations, the disintegrations per minute of plasma deoxyglucose over time, and the global or regional optical densities for the brain, cerebral glucose utilization (or metabolic rate, in μmol/100 gm/min) was calculated using the equation of Sokoloff et al. (1977). All of the regional 2DG data were then subjected to a 2-way analysis of variance for repeated measures (brain structures) to ascertain whether significant differences occurred in glucose utilization between the 2 sides of the brain. Since utilization was similar bilaterally, these data were averaged to produce one value for each brain structure per rat. Four repeat measure analyses of variance (ANOVA) were then performed on the averaged 2DG data. In 3 of the ANOVAs, the grouping factor included the saline controls, one of the models, one of the models plus tranylcypromine, and one of the models plus another antidepressant) to be discussed in another paper) per ANOVA. In the fourth ANOVA, the grouping factor included tranylcypromine alone (given via 2 different injection protocols), the other antidepressant, and the controls. The repeated factor for all ANOVAs were the various brain structures. Since some of the data were used in more than one ANOVA, Bonferroni corrections for multiple comparisons were applied to these ANOVAs (to be conservative, $p < 0.005$ was required for significance). When an analysis of variance was significant, *post hoc* Newman-Keuls tests were used to obtain information regarding differences in metabolic rates between individual brain sites. When an analysis of variance was not significant, individual differences in metabolic rates between brain structures were determined using a Bonferroni t statistic. The forebrain global 2DG data were analyzed using a 1-way ANOVA between the experimental groups; t tests were then used to obtain information about individual experimental groups.

Results

Behavioral studies

Eleven groups of rats were used in the behavioral studies. Three groups received the AMPT, Amp WD, or Chronic stress models, respectively; 3 other groups received the models plus the antidepressant drug tranylcypromine, 2 groups received tranylcypromine alone (via different injection protocols), and 3 groups served as handled controls to each of the model groups. Separate groups were used because each rat was tested in the open field only once so as to preserve the novelty of the experience. While in the open field, each rat was rated for the number of squares crossed and the number of rears performed during a 6 min testing session.

The 3 control groups did not differ in the number of squares that they crossed; thus, these data were averaged together. In contrast, a significant difference was found in the number of squares crossed between control rats and rats given the models alone, models-plus-tranylcypromine, or tranylcypromine alone (ANOVA, $F = 11.44$, $p < 0.0001$). Rats given any of the 3 models (AMPT, Amp WD, or Chronic stress) crossed significantly fewer squares than control rats ($p < 0.05$, Newman-Keuls test, for each model/control comparison). As can be seen in

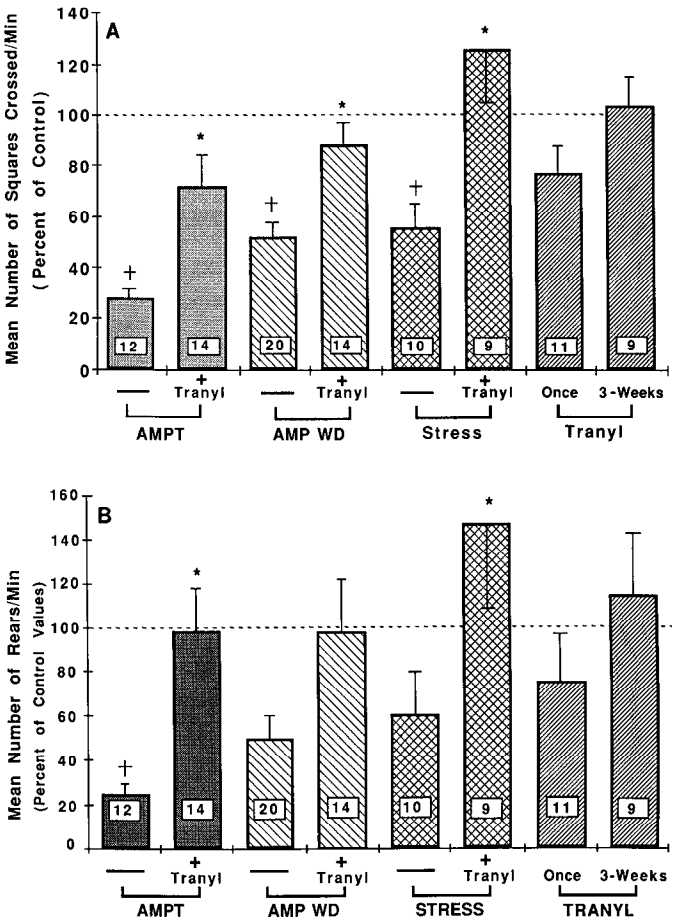


Figure 1. Locomotion (square crossing; *A*) and rearing (*B*) in an open field in 3 models of depressed behavior. The α-methyl-para-tyrosine (*AMPT*), amphetamine withdrawal (*AMP WD*), and Stress models were each tested alone and in combination with the antidepressant tranylcypromine (*+tranyl*). Data from models alone and in combination with tranylcypromine are given as percentages of control values. Tranylcypromine was given as a single injection in the AMPT and Amp WD models and for 3 weeks in the Stress model. Numbers within the bars indicate the number of rats per group. +, significant differences from controls; *, significant differences from the same model without antidepressant drugs.

Figure 1*A*, the AMPT model produced the most severe reductions in squares crossed (73% reduction from control values), the Amp WD model resulted in lesser reductions (49% reduction from controls), and the Stress model showed the smallest reductions (45% reduction from controls). Tranylcypromine, by itself, given either as 1 injection or for 3 weeks, did not produce significant differences in the number of squares crossed compared with control rats. However, tranylcypromine, given in conjunction with each of the models, significantly prevented or minimized the reductions in the number of squares crossed seen with the models alone (Fig. 1*A*).

Unlike the squares data, the number of rears performed by the 3 groups of control rats differed among the groups ($F = 3.95$, $p < 0.03$, ANOVA). Data for each of the 3 control groups were thus compared with data only from each corresponding model. A significant difference was observed in the number of rears performed by controls compared with rats given the models alone, the models plus tranylcypromine, or tranylcypromine alone ($F = 5.70$, $p < 0.0001$). While the number of rears performed was reduced in each of the models compared with con-

Table 1. Glucose metabolism (μmol/100 gm/min) in control and AMPT rats (means ± SE)

Brain structure	Control (N = 13) Left brain	Right brain	AMPT (N = 7) Left brain	Right brain
Dorsal medial prefrontal cortex	132.9 ± 3.6	129.5 ± 3.6	88.8 ± 5.6	88.8 ± 5.5
Motor cortex	116.5 ± 3.2	118.8 ± 3.2	91.7 ± 4.6	91.9 ± 4.7
Accumbens	98.9 ± 4.5	94.7 ± 3.2	80.6 ± 8.9	79.8 ± 8.9
Corpus callosum	33.2 ± 1.3	31.9 ± 1.1	25.8 ± 2.1	25.4 ± 1.1
Caudate nucleus	116.4 ± 3.9	112.9 ± 4.2	89.0 ± 5.5	90.6 ± 6.4
Cingulate cortex	124.1 ± 4.7	124.0 ± 4.3	93.3 ± 4.8	92.0 ± 4.8
Lateral septum	66.0 ± 2.4	65.8 ± 2.4	49.3 ± 6.0	51.0 ± 7.0
Anterior ventral thalamus	108.2 ± 3.6	109.4 ± 3.8	73.3 ± 3.5	73.3 ± 3.3
Medial forebrain bundle	67.4 ± 1.5	65.7 ± 1.5	51.8 ± 4.1	52.0 ± 4.9
Basolateral amygdala	83.7 ± 3.0	77.7 ± 6.3	65.5 ± 3.6	67.7 ± 3.3
Lateral habenula	108.9 ± 2.1	109.4 ± 1.8	138.6 ± 9.5	138.5 ± 11.1
Ventral medial hypothalamus	57.1 ± 2.3	57.0 ± 2.3	49.3 ± 3.7	47.9 ± 3.1
Pyriform cortex	66.2 ± 2.5	64.3 ± 2.2	57.9 ± 5.1	59.2 ± 4.5
Dorsal hippocampus pyramidal	86.8 ± 2.0	88.0 ± 2.2	67.6 ± 3.9	67.3 ± 3.5
Ventral tegmental area	61.0 ± 2.2	60.0 ± 1.9	47.9 ± 2.4	50.2 ± 2.8
Substantia nigra	59.9 ± 1.3	58.9 ± 1.1	46.2 ± 3.8	48.2 ± 4.5
Ventral hippocampus pyramidal	87.2 ± 3.3	85.1 ± 3.0	67.7 ± 4.1	67.6 ± 3.9
Dentrate hilus	64.4 ± 2.0	62.3 ± 1.8	56.2 ± 4.3	56.2 ± 4.9
Medial geniculate	130.3 ± 6.1	130.0 ± 4.8	98.4 ± 7.6	102.4 ± 8.5
Interpeduncular nucleus	Not measured	98.3 ± 3.4	Not measured	81.0 ± 3.4
Reticular formation	63.5 ± 2.1	62.2 ± 2.2	48.8 ± 2.4	48.8 ± 2.6
Periaqueductal gray	Not measured	73.6 ± 1.5	Not measured	55.4 ± 2.6
Entorhinal/subiculum	78.9 ± 2.8	77.4 ± 2.1	61.3 ± 3.5	62.1 ± 3.7
Inferior colliculus	175.5 ± 10.0	182.0 ± 7.5	145.4 ± 10.8	148.3 ± 8.0
Nucleus tegmentalis dorsalis	108.3 ± 6.1	106.6 ± 5.6	73.9 ± 2.7	74.2 ± 2.1

trols (Fig. 1*B*), this reduction was significant only in the AMPT model ($p < 0.05$, Newman-Keuls). Tranylcypromine alone (1 injection or for 3 weeks) did not significantly alter the number of rears performed compared with control rats. However, tranylcypromine significantly prevented the reduction of rearing seen in the AMPT alone group ($p < 0.05$, Newman-Keuls, Fig. 1*B*). Tranylcypromine, in combination with the stress model, also significantly increased the amount of rearing compared with Stress alone ($p < 0.05$, Newman-Keuls).

Regional 2DG studies

Nine groups of rats were used in the 2DG autoradiographic studies. Three groups received the AMPT, Amp WD, or Chronic stress models, respectively, 3 other groups received the models plus tranylcypromine, 2 groups received tranylcypromine alone, and one group served as controls. As discussed in the introduction, changes in the rates of glucose metabolism were considered as true correlates of the depressed behaviors only if the rate changes were significant and in the same direction in each of the 3 models. Therefore, the following results and discussion sections emphasize these similar changes in metabolic rates, although all of the metabolic data are included in the ANOVA and are shown in the figures.

A preliminary ANOVA established that regional glucose metabolic rates for the 2 sides of the brain did not differ significantly. (The similarities in left/right metabolic rates are illustrated in Table 1.) All figures and subsequent statistical tests therefore used the averaged metabolic rates for each brain structure. The variability of the data is not shown in the figures (for clarity) but is illustrated in Table 1.

A subsequent ANOVA revealed that significant differences exist between regional rates of glucose metabolism in rats given the AMPT model alone, AMPT plus tranylcypromine, AMPT plus another antidepressant (discussed in another paper), and controls ($F = 5.46$, $p < 0.004$). Likewise, rats given the Amp WD model alone, the Amp WD model plus tranylcypromine, Amp WD plus another antidepressant, and controls also differed in their regional rates of glucose metabolism ($F = 5.52$, $p < 0.004$). However, a similar ANOVA on the metabolic rates for the Stress model revealed no significant differences from controls. These overall results are reflected in the amount of separation between the graphs for each model and the graph of the controls (Fig. 2*B*). The rate of glucose metabolism in AMPT animals was, overall, most different from controls. Metabolic rates in Amp WD rats were less different from controls, and stressed rats were the least different from controls.

Most importantly, *post hoc* Newman-Keuls and Bonferroni t tests demonstrated significant changes in the glucose metabolic rates of specific brain sites in each of the models compared with controls. Elevations ($p < 0.05$) in glucose metabolic rates, compared with controls, were found in all 3 models in the lateral habenula, LHAB (Fig. 2, *A, B*). Also, decreases ($p < 0.05$) in metabolic rates occurred in all 3 models in the dorsal medial prefrontal cortex (DMFC), the anterior ventral nucleus of the thalamus (AVT), and the inferior colliculus, compared with controls (Fig. 2*B*). Additional brain sites showed significant decreases in metabolic rates compared with controls, but these changes were not consistent across all models. The affected structures included the dorsal tegmental nucleus, caudate nucleus, and the motor cortex in the AMPT and Amp WD models,

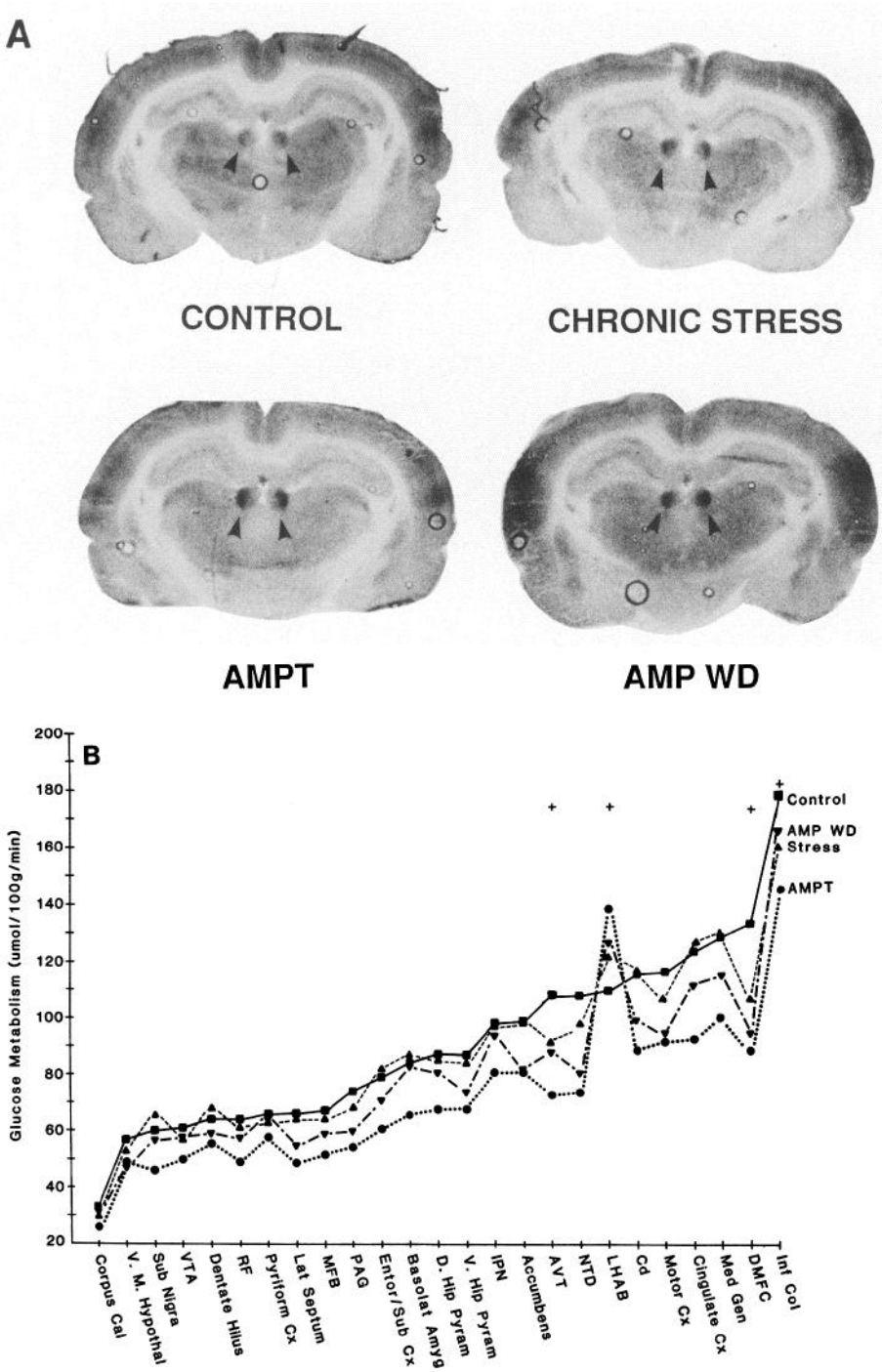


Figure 2. Regional glucose metabolic rates in each of the 3 models and controls. *A,* Autoradiograms showing increased rates of metabolism in the lateral habenula (*arrows*). *B,* Glucose metabolic rates (μmol/100 gm/min) for 25 brain sites. Values are averaged from the left and right sides of the brain. $N =$ 13 (control), 10 (AMP WD), 11 (Stress), 7 (AMPT); +, significant differences in all 3 models from controls. Abbreviations of brain sites are as follows: *Corpus Cal,* corpus callosum; *V.M. Hypothal,* ventral medial hypothalamus; *Sub Nigra,* substantia nigra; *VTA,* ventral tegmental area; *RF,* reticular formation; *Pyriform cx,* pyriform cortex; *Lat Septum,* lateral septum; *MFB,* medial forebrain bundle; *PAG,* periaqueductal gray; *Entor/Sub cx,* entorhinal and subicular cortex; *Basolat Amyg,* basolateral amygdala; *D. Hip. Pyram,* pyramidal cell layer of the dorsal hippocampus; *V. Hip. Pyram,* pyramidal cell layer of the ventral hippocampus; *IPN,* interpeduncular nucleus; *AVT,* anterior ventral nucleus of the thalamus; *NTD,* dorsal tegmental nucleus; *LHAB,* lateral habenula; *Cd,* caudate; *Motor Cx,* motor cortex; *Med Gen,* medial geniculate; *DMFC,* dorsal medial prefrontal cortex; *Inf Col,* inferior colliculus.

as well as the medial geniculate and cingulate cortex in the AMPT model (significance not shown in figures).

Tranylcypromine prevented or minimized several of the changes in the rates of glucose metabolism caused by the models alone. In the AMPT and Amp WD models, glucose metabolic rates were significantly ($p < 0.05$) lower in the lateral habenula and higher in the inferior colliculus in rats given tranylcypromine plus the models compared with those given the models alone (Figs. 3, *A, B*; 4, *A, B,* respectively). In the Stress model, glucose metabolic rates were lower ($p < 0.05$) in the LHAB of rats given tranylcypromine plus the model compared with the model alone (Fig. 5, *A, B*). In contrast, tranylcypromine given by itself for 3 weeks or as a single injection did not significantly affect rates of glucose metabolism compared with control animals (ANOVA and Bonferroni *t* statistics; Fig. 6).

Forebrain global 2DG studies

The same model and control groups were used in these studies as in the regional 2DG studies. Forebrain global metabolic rates (including the telencephalon and diencephalon minus the hypothalamus and medial geniculates) were significantly different across the control, model, model plus tranylcypromine, and tranylcypromine-alone groups ($F = 2.72$, $p < 0.01$, ANOVA). All 3 models of depressed behavior produced significant reduc-

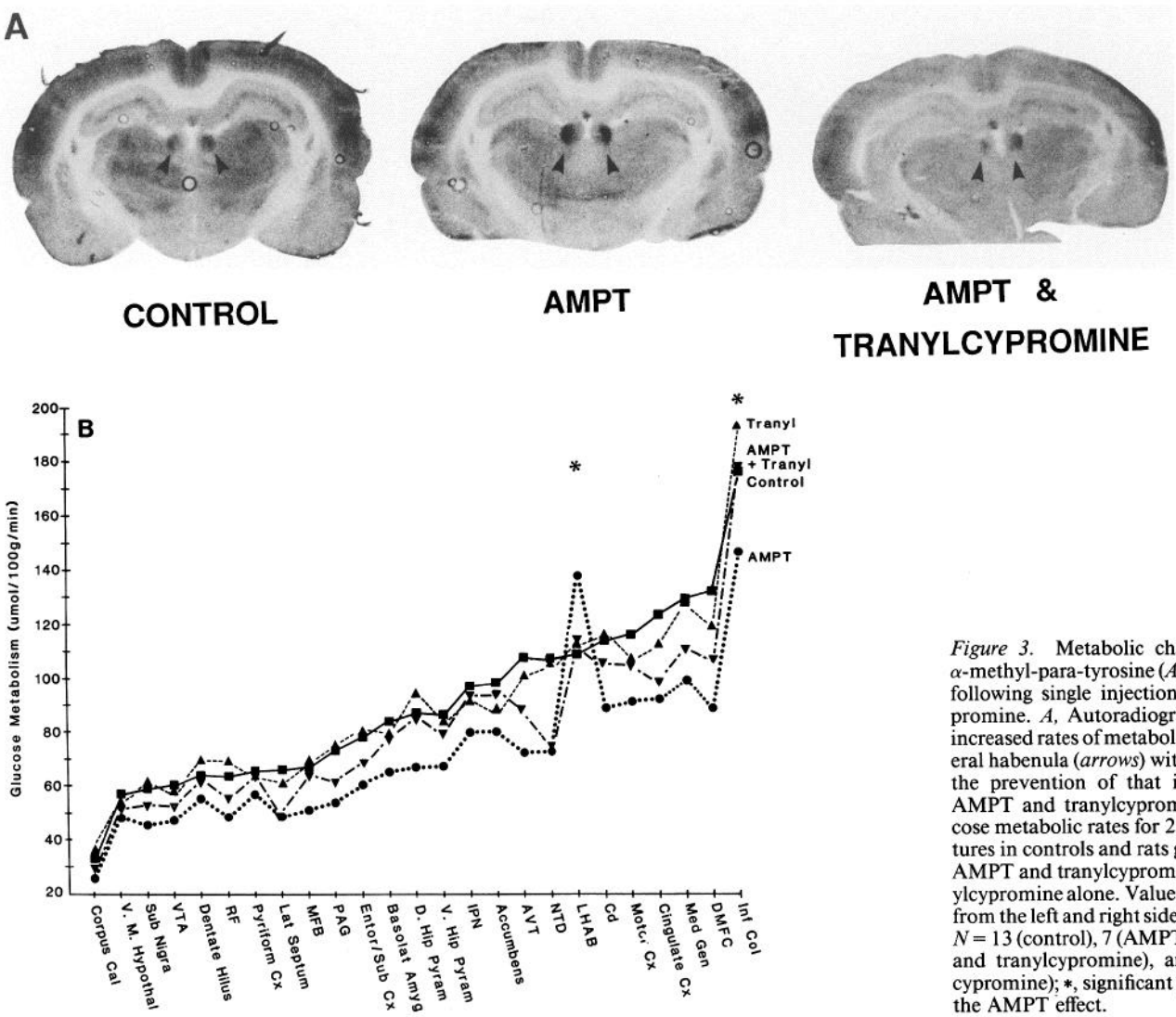


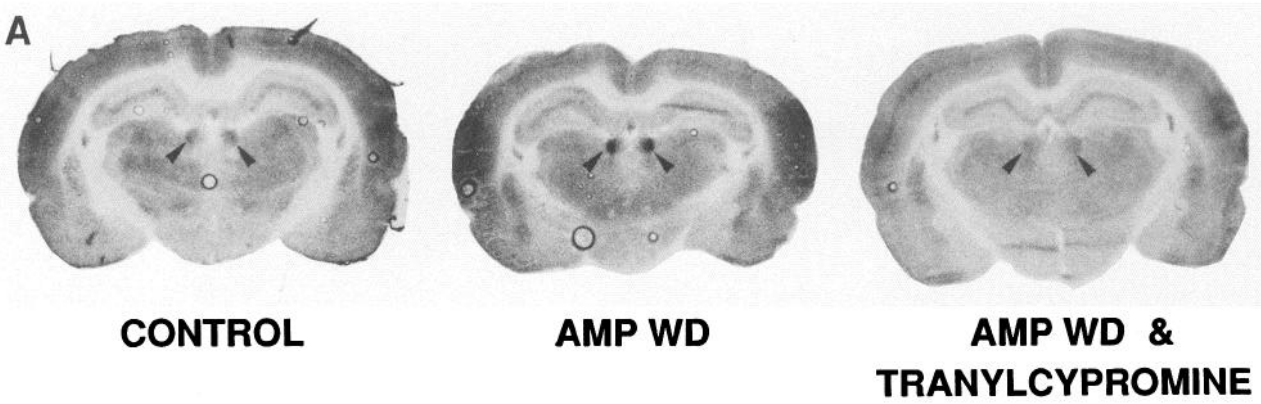
Figure 3. Metabolic changes in the α-methyl-para-tyrosine (*AMPT*) model following single injection of tranylcypromine. *A,* Autoradiograms showing increased rates of metabolism in the lateral habenula (*arrows*) with AMPT and the prevention of that increase with AMPT and tranylcypromine. *B,* Glucose metabolic rates for 25 brain structures in controls and rats given AMPT, AMPT and tranylcypromine, and tranylcypromine alone. Values are averages from the left and right sides of the brain. $N = 13$ (control), 7 (AMPT), 12 (AMPT and tranylcypromine), and 5 (tranylcypromine); *, significant prevention of the AMPT effect.

tions in forebrain global metabolism compared with controls (Fig. 7; $p < 0.05$, t tests for each model/control comparison). The greatest reduction in metabolism, 33% from controls, occurred in the AMPT model; lesser reductions occurred in the Chronic stress model (16% from controls) and in the Amp WD model (15% from controls). Tranylcypromine, given by itself, either as a single injection or for 3 weeks did not significantly alter forebrain global metabolic rates compared with controls. Likewise, the forebrain global rates in rats given one injection of tranylcypromine did not significantly differ from the rates when the drug was given for 3 weeks. When given in combination with the AMPT and Stress models, tranylcypromine partially prevented the reductions in metabolism seen in the AMPT and Stress models alone (Fig. 7). However, while metabolism in these 2 models given tranylcypromine was not significantly different from that in controls, the differences in metabolism between these models alone and these models given tranylcypromine was also not significant. In the Amp WD model, tranylcypromine had no effect on the reduced metabolism seen in the model alone.

Discussion

Behavioral studies

The AMPT, Amp WD, and Stress models of depressed behavior used in our study were copied from previous reports in the literature. Not only had these rat models been reported to mimic several features of human depression (Poschel and Ninteman, 1966; Rech et al., 1966; Moore and Rech, 1967; Lynch and Leonard, 1978; Kokkinidis et al., 1980; Leith and Barrett, 1980; Katz et al., 1981a; Cahill and Ehret, 1982; Katz, 1982; Katz and Baldrighi, 1982), but AMPT, Amp WD, and Stress were also known to induce depression in humans (Watson et al., 1972; Ainsman and Zacharko, 1982; Gillin et al., 1985). Thus, by using these models, we benefited from the information already obtained about them, and we avoided the time that had been necessary to develop them. The behaviors that we tested as part of the models, square crossing and rearing in an open field, were also taken from the literature. Although these rat behaviors may seem simplistic, experiments have indicated that they probably measure emotionality (Hall, 1934, 1936; Broadhurst, 1957) and/



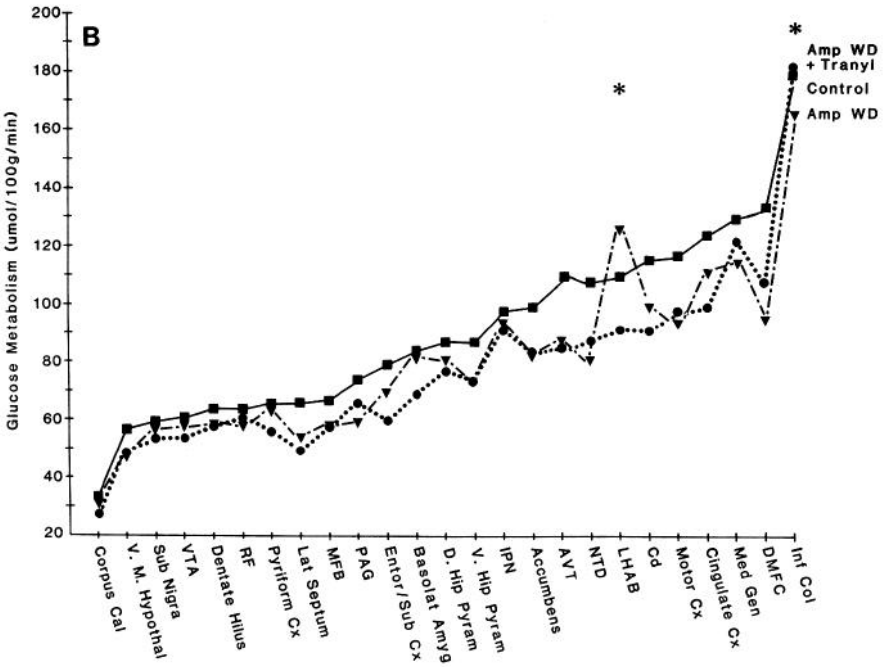


Figure 4. Metabolic changes in the amphetamine withdrawal (*AMP WD*) model following single injection of tranylcypromine. *A,* Autoradiograms showing increased rates of metabolism in the lateral habenula (*arrows*) with Amp WD and the prevention of that increase with Amp WD and tranylcypromine. *B,* Glucose metabolic rates for 25 brain structures in controls and rats given Amp WD or Amp WD and tranylcypromine. Metabolic rates in rats given tranylcypromine alone were the same as in Figures 3 and 6. Values are averages from the left and right sides of the brain. $N = 13$ (control), 10 (Amp WD), and 6 (Amp WD and tranylcypromine); *, significant prevention of the Amp WD effect.

or exploration in response to the novelty of an open field (Whimbey and Denenberg, 1967; Fink and Smith, 1979). Thus, square crossing and rearing probably have psychological as well as motor components, and they can be considered psychomotor behaviors. Furthermore, if reductions in these behaviors in rats are not due to motor deficits, then such reductions may be similar to psychomotor retardation in depressed humans. The following data suggest that our results were not due to motor deficits. While square crossing was decreased in all models, other motor behaviors, rearing and grooming, were not significantly reduced in our Stress and Amp WD models. Grooming was also unaffected in the AMPT model. In addition, preliminary results in the AMPT model showed that bar pressing to administer rewarding, electrical brain stimulation was reduced overall, but was as rapid, during various 1 min periods, as in control animals. This rapid responding, plus the presence of locomotion in these animals during reduced bar pressing, indicated that the motivation to obtain rewarding effects of the stimuli was diminished, not the motor skills needed to push the bar.

Reduced exploration by our rats may also correspond to known reductions in behavioral (Kaplan and Sadock, 1985) and physiological (Litzelman et al., 1980) responding to novel stimuli in depressed patients. Furthermore, reduced responding to novel stimuli in both species may correlate with decreased functioning of the frontal cortex. For example, the P300 event-related potential (thought to represent brain response to novel stimuli) has a smaller amplitude over the frontal cortex in depressed than in nondepressed patients (Litzelman et al., 1980). Humans with physical damage to the prefrontal cortex also show reductions in the amplitude of the P300 wave over the frontal cortex (Knight, 1984). Finally, the rate of glucose metabolism in the frontal cortex is reduced in bipolar depressed patients (Baxter et al., 1985). If reductions in metabolic rate reflect deafferentation of that brain region (Ackermann et al., 1984), then regions with decreased metabolic rates may be hypofunctional. The data from our rat models of depressed behavior, showing reductions of both exploratory behavior and metabolic rates in the prefrontal cortices, thus also support a relationship between reduced cortical functioning and decreased novelty responding.

Our demonstration of reductions in square crossing in the AMPT, Amp WD, and Stress models, compared with controls, supports the studies from which our models were copied (Rech

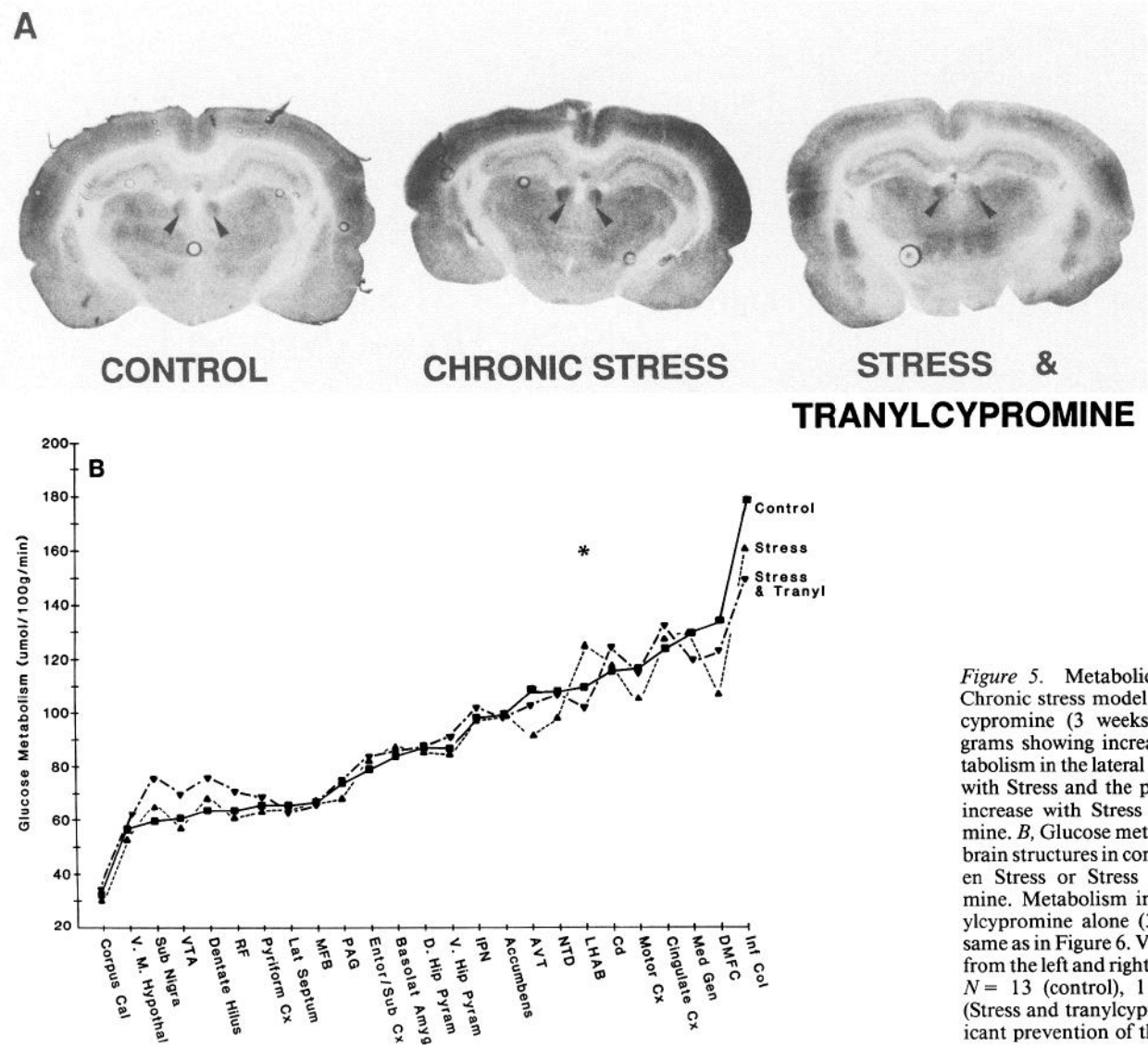


Figure 5. Metabolic changes in the Chronic stress model following tranylcypromine (3 weeks). *A,* Autoradiograms showing increased rates of metabolism in the lateral habenula (*arrows*) with Stress and the prevention of that increase with Stress and tranylcypromine. *B,* Glucose metabolic rates for 25 brain structures in controls and rats given Stress or Stress and tranylcypromine. Metabolism in rats given tranylcypromine alone (3 weeks) was the same as in Figure 6. Values are averages from the left and right sides of the brain. $N = 13$ (control), 11 (Stress), and 5 (Stress and tranylcypromine); *, significant prevention of the Stress effect.

et al., 1966; Lynch and Leonard, 1978; Katz et al., 1981a). Likewise, our results that tranylcypromine prevented the reduction of square crossing behavior in each model replicated the tranylcypromine-induced prevention of decreased locomotion in previous AMPT (Moore and Rech, 1967) and Stress models (Katz et al., 1981b). Our finding of no significant change in rearing in the Amp WD and Stress models was in contrast to the significant effects of these models on rearing in other studies (Lynch and Leonard, 1978; Katz et al., 1981a). The large degree of variability in our rearing data may account for these discrepancies.

2DG studies

In each of our models of depressed behavior, the rate of glucose metabolism was elevated in the LHAB and was reduced in the DMFC, AVT of the thalamus, and the inferior colliculus compared to controls. Since these changes in metabolic rate occurred in each of the models, the changes were considered to be true correlates of depressed behavior. Tranylcypromine also prevented the elevation of metabolic rate in the LHAB in each of the models. Thus, as in the models alone, tranylcypromine's effects correlated with a generalized behavior state, rather than being caused by an idiosyncrasy of one model.

The effects of AMPT, withdrawal from amphetamine, or stress on cerebral rates of glucose metabolism have not previously been reported. However, amphetamine's effects on metabolism have been reported (Wechsler et al., 1979). Interestingly, reductions in the rate of glucose metabolism in the LHAB during amphetamine administration (Wechsler et al., 1979) were consistent with our findings of an elevation in metabolism in the same structure during withdrawal from amphetamine (as well as during the administration of AMPT and stress).

Furthermore, the correlation of metabolic changes in the LHAB, prefrontal cortex, and AVT with depressed behavior corroborates previous reports that these structures may mediate emotional behaviors in animals and humans. For example, either electrical stimulation or morphine injections into the LHAB produce analgesia to painful stimuli (Benabid and Mahieux, 1984; Cohen and Melzack, 1985). Also, LHAB lesions prevent antidepressant drugs from reversing depressed behavior, which is induced by forced swimming (Thornton et al., 1985). Changes in frontal cortex imipramine receptors have been observed dur-

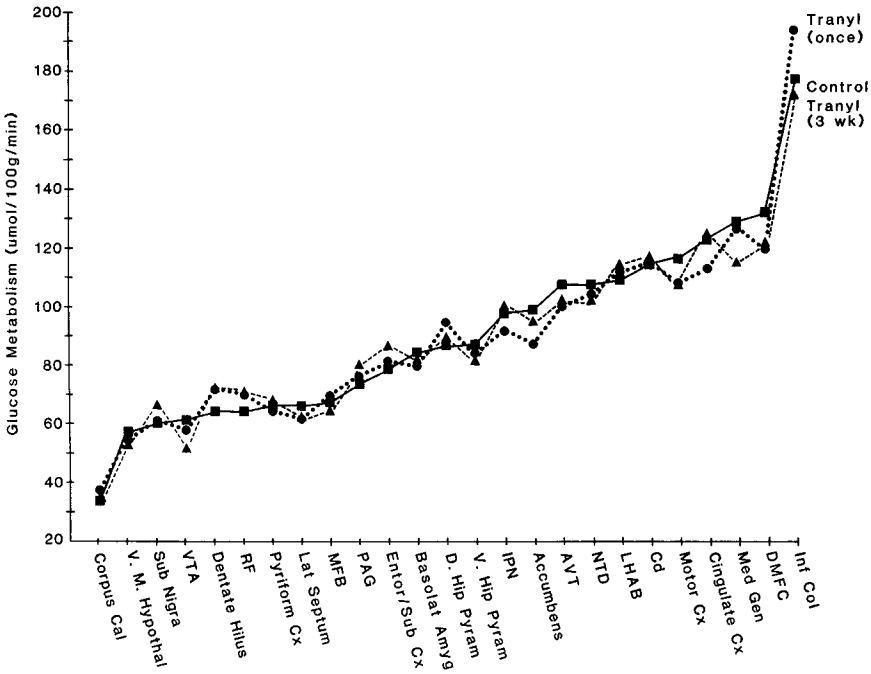


Figure 6. Metabolic changes in rats given tranylcypromine. Glucose metabolic rates for 25 brain structures in controls and rats given tranylcypromine once—*Tranyl(once)*—or for 3 weeks—*Tranyl(3 wk)*. Values are averages from the left and right sides of the brain. $N = 13$ (control), 5 (tranylcypromine once), and 5 (tranylcypromine 3 weeks).

ing depressed behavior caused by learned helplessness in rats (Johnson et al., 1982). Frontal lobe reductions in the rate of glucose metabolism (Baxter et al., 1985) and in the amplitude of the P300 wave (Litzelman et al., 1980) also correlate with depression and a decrease in novelty responding in humans. Other human studies of glucose metabolism during painful stimulation (Buchsbaum et al., 1984) and postmortem receptor binding (Stanley and Mann, 1983; Zanko and Biegon, 1983) have found changes in the frontal cortex correlating with depressed behavior. Finally, patients with bipolar depression show reductions in glucose utilization in the thalamus, although individual nuclei could not be resolved (Baxter et al., 1985).

Since metabolic changes in the LHAB, prefrontal cortex, and AVT occur simultaneously, one wonders whether these structures are part of a circuit that mediates depressed behavior. Anatomical evidence exists that such a circuit could be present. The prefrontal cortex, LHAB, and AVT are all limbic structures (Papez, 1937; Kaada, 1960; Truex and Carpenter, 1969; Sutherland, 1982; Seki and Zyo, 1984) that are interconnected by mono- or disynaptic pathways. The AVT receives direct projections from the LHAB (Sutherland, 1982) and from the prefrontal cortex (Lisoprawski et al., 1980). The LHAB also sends efferents to (Phillipson, 1978; Herkenham and Nauta, 1979) and modifies the firing of ventral tegmental area dopamine cells that project to the prefrontal cortex (Lisoprawski et al., 1980). The prefrontal cortex projects directly to the LHAB through a dorsal route traversing the anterior medial and mediodorsal nuclei of the thalamus (Beckstead, 1979). The prefrontal cortex also sends efferents to the septum, which projects via the stria medullaris to the LHAB (Nauta and Haymaker, 1969; Sutherland, 1982). This latter pathway is part of a dorsal diencephalic conduction system, which carries information from forebrain to midbrain limbic areas, and is hypothesized to function in parallel with the primary limbic pathway, the medial forebrain bundle (Sutherland, 1982). Thus, the pathways connecting all of these structures could allow for interstructure modulation of function, perhaps resulting in the metabolic changes observed during reduced exploratory behavior.

In addition to these anatomical data, some physiological and pharmacological data also support the participation of interstructure interactions in the mediation of depressed behavior. For example, hypermetabolism, as in the LHAB of our depression models, is thought to reflect an increase in synaptic and/or cellular activity (Ackermann et al., 1984). Furthermore, activation of the habenula by electrical stimulation has been shown to inhibit the firing of dopamine-containing cells in the ventral tegmental area (VTA; Christoph et al., 1986). Also, lesions of the VTA decrease the number of dopamine-containing fibers that innervate the medial prefrontal cortex (Tassin et al., 1978). A decrease in the innervation of a brain structure is thought to

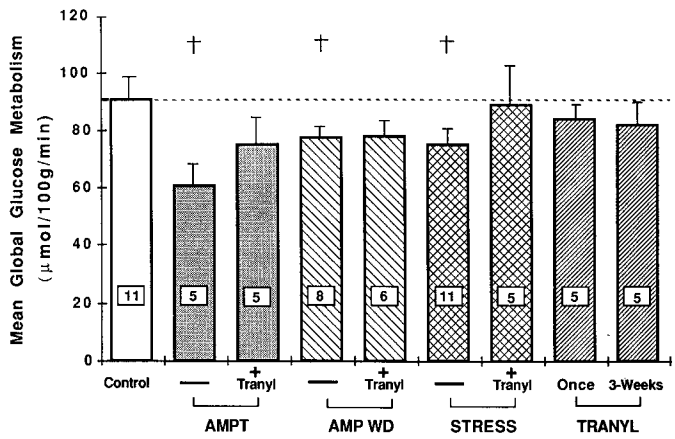


Figure 7. Global metabolic rates (μmol/100 gm/min) in 3 models of depressed behavior. Metabolic rate was measured in each model alone (*AMPT, AMP WD,* and *STRESS*) and in combination with tranylcypromine (+*Tranyl*). Tranylcypromine was given as a single injection in the AMPT and Amp WD models and for 3 weeks in the Stress model. Numbers within the bars indicate the number of rats per group; +, significant difference from controls.

reduce the rate of glucose metabolism in that structure (Ackermann et al., 1984). Thus, some of our metabolic results could be explained by increased activity (causing hypermetabolism) in the LHAB of our depression models, which would inhibit VTA cells and decrease activity at the terminals of the prefrontal cortex, causing hypometabolism in that structure.

Our hypothesized decrease in the innervation of the prefrontal cortex (and hence of dopamine utilization) during stress and drug-induced depressed behavior appears to contradict previous data. Specifically, Thierry et al. (1976) reported that stress increased dopamine utilization in the prefrontal cortex. However, this increase in dopamine utilization was observed after 20 min of acute stress, whereas our depressed behavior was measured after 3 weeks of prolonged stress. Prolonged and acute stress may activate different neural systems, which could produce different rates of glucose metabolism. Previous studies have supported this idea. For example, Soblosky and Thurmond (1986) reported that acute stress produced an elevation of whole-brain 5-HT levels, whereas chronic stress elevated only whole-brain norepinephrine levels.

Brain structures other than the LHAB, prefrontal cortex, AVT, and inferior colliculus may also influence the production of depressed (reduced exploratory) behavior in our models. This idea is suggested by the conflicting results of 2 studies. Fink and Smith (1979) showed that reduced short-term (10 min) exploratory behavior correlated with a decrease in the number of dopamine-containing cell bodies in the VTA and substantia nigra and with decreased dopamine terminals in the prefrontal cortex and other cortical and subcortical limbic structures. These results were produced by lesions of the dopamine-containing fibers in the anterolateral hypothalamus. On the other hand, a direct lesion of the dopamine-containing cell bodies in the VTA, which also project to the prefrontal cortex and other cortical and subcortical areas, produces no apparent effect on short-term locomotion in a novel environment and increases locomotion when measured over 12 hr (Le Moal et al., 1969). The disparate results of these 2 studies are probably due to different effects of the lesions on structures participating in the mediation of depressed exploratory behavior. While the LHAB, AVT, and inferior colliculus were not analyzed in these studies, different effects on these structures or on other brain areas could have influenced behavior. Our demonstration of decreases in global metabolic rates in the forebrain of all of our depression models also supports the idea that additional structures contribute (though perhaps less significantly on an individual basis) to the mediation of depressed behavior. Furthermore, our forebrain global metabolic results correlate well with the behavioral (square crossing) results. The largest reductions in both measures occurred in the AMPT model (Figs. 1*A* and 7). The reductions in behavior and forebrain global metabolism were similar in the Amp WD and Stress models, and both were less than in the AMPT model.

Our global metabolic data are also similar to the results of human studies in which supratentorial global glucose metabolism is reduced concomitant to the depressed phase of bipolar affective disorders (Baxter et al., 1985). The brains of the depressed patients showed supratentorial global reductions in metabolism of 29% compared with controls. In our animal studies, forebrain global reductions of 33% in the AMPT model were especially close to the human data. However, unlike the data in patients with bipolar disorders (where euthymic or hypomanic states coincided with normalization of supratentorial global brain metabolism; Baxter et al., 1985), the reduced forebrain global brain metabolism in our animal models was not significantly prevented by tranylcypromine. While a trend toward greater rates of metabolism occurred in the AMPT and Stress groups, a large variance in the data may have prevented these effects from reaching significance.

In summary, the present report establishes that similar, reproducible alterations in regional and forebrain global brain glucose metabolism can be obtained following the administration of AMPT, Amp WD, or Chronic stress in rats. Furthermore, these metabolic changes correlate with reductions in behavior that are induced by the same preparations. An antidepressant drug, tranylcypromine, prevents some of the metabolic changes as well as the behavioral changes. And, most interestingly, many of the metabolic and behavior changes parallel human studies of cerebral metabolism in bipolar depression, thereby supporting a claim that the AMPT, Amp WD, and Stress preparations serve as models for human depressed behavior.

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