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Rats selectively-bred for behavior related to affective disorders: Proclivity for intake of alcohol and drugs of abuse, and measures of brain monoamines

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ABSTRACT

Several lines of rats potentially useful for studying affective disorders have been developed in our laboratory though selective breeding for behavioral characteristics. The propensity of these lines to consume alcohol and other drugs of abuse (amphetamine and cocaine) was examined. Also, measurement of the concentration of brain monoamines – norepinephrine, dopamine, and serotonin – as well as estimation of their metabolism by measurement of the major extracellular metabolites of these monoamines was carried out to examine possible relationships of brain chemistry to the behavioral characteristics shown by these lines, as well as to their propensity for drug usage. The lines of rats are: Swim Low-active (SwLo) and Swim High-active (SwHi), which show either very low (SwLo) or very high (SwHi) amounts of motor activity in a swim test; Swim-test Susceptible (Susceptible or SUS) and Swim-test Resistant (Resistant or RES), which are highly susceptible (SUS) or highly resistant (RES) to having their swim-test activity depressed by being exposed to a stressful condition prior to the swim test; and Hyperactive (HYPER), which show spontaneous nocturnal hyperactivity compared to non-selectively bred (i.e., normal) rats as well as both extreme hyperactivity and behavioral depression after being exposed to a stressful condition. Regarding alcohol and drug usage, SUS rats readily consume alcohol while all other lines including non-selected, normal rats do not, and SwLo rats show a strong tendency to consume amphetamine and cocaine. Marked differences in brain monoamines were found between the various lines and normal rats, with salient differences seen in norepinephrine, particularly in the hippocampus, and in dopamine in forebrain regions (striatum and nucleus accumbens).

1. Introduction

In this paper, we will describe the origin and development of several lines of Sprague–Dawley rats that have been derived through selective breeding, describe some recent findings related to the propensity of these animals to consume alcohol or addictive drugs, and, finally, present findings related to differences in brain monoamine content and metabolism in these different rat lines.

2. Selectively-bred rat lines

In an effort that began in the early 1980s, we undertook to develop different rat lines in the hope of producing improved rat models for the study of affective disorders. These rat lines were generated by selective breeding, a process in which animals are chosen for breeding because they show particular characteristics. For the lines we have developed, rats were chosen for breeding based on behavioral characteristics.

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These lines were directed at the possibility of improving both drug screens (i.e., improved sensitivity and selectivity of detection of antidepressant drugs) and "homologous" models of depression (i.e., rats that show characteristics seen in human depression) (see Weiss and Kilts [1] for further explanation of these categories). In this effort, we were influenced by years of having studied a phenomenon called "learned helplessness" or "stress-induced behavioral depression" (summarized in Maier and Seligman [2] and Weiss et al. [3] and Weiss [4]) in which we consistently observed that (a) depression-like symptoms appeared only in some, but certainly not all, of the animals exposed to stressful conditions and then tested, and (2) even in animals that showed appropriate symptoms, the symptoms were too short-lived (i.e., lasting 48–96 h) to qualify as successfully modeling depression seen in humans. This led to our thinking that adequate modeling of depression was unlikely to accomplished using "normal" animals; rather, this type of response might instead derive from a vulnerability present only in specific individuals in the population. This would seem to parallel the human situation in that people afflicted by severe forms of affective disorders apparently are a subset of the total population, which is evident from the observation that genetic factors influence susceptibility of people to many forms of major psychiatric disturbance. There is considerable evidence indicating that humans show genetic predisposition to depression (e.g., [5–10]). For this reason, we attempted to selectively breed rats that might have appropriate predispositions. The rat lines that have been developed are described below:

2.1. *Swim low-active (SwLo) and Swim high-active (SwHi) rats*

Amongst the first selectively-bred lines we attempted to develop were rats that would show, at "baseline" without any stress or additional provocation, high or low activity in a swim test. In 1977, Roger Porsolt and his colleagues published a description of what has become the most widely-used preclinical screening technique for detecting effective antidepressant treatments—the swim test (Porsolt et al. [11]). Stated simply, effective antidepressant treatments increase activity of rodents in a swim test. In the usual application of the test to rats, animals are introduced into the swim test on the first day, and remain in the swim tank for 15 min. During this time, their active coping attempts (active behavior in the swim test) diminish, so that the animal is relatively inactive at the end of this exposure to the swim tank. When re-exposed to the swim tank 24 h later, animals therefore begin the test with a "learned" tendency to be inactive. It is this tendency to be inactive that effective antidepressants tend to counteract. The test is highly useful, detecting a wide variety of antidepressants treatments, but it had some notable shortcomings, particularly the fact that this test was found to be relatively unresponsive to selective serotonin reuptake inhibitors (SSRIs) despite occasional successes in this regard [12–15]. We reasoned that if we generated, by selective breeding, animals that show low activity in the swim test as a genetically-determined characteristic, perhaps these animals would be better subjects for detection of antidepressant treatments than were normal rats that had to "learn" to be inactive.

Therefore, we selected from a large population of normal animals (i.e., a foundation stock of 84 male and 48 female Sprague–Dawley rats purchased from Charles River Breeding Laboratories in 1987) those animals (both males and females) that showed the least activity in the swim test, and repeatedly bred these animals over succeeding generations. Having introduced scoring of active escape-directed behavior for the swim test (i.e., "struggling" behavior) as well as quantification of immobility (i.e., "floating" behavior) some years earlier (Weiss et al. [16]), we chose for breeding those animals that showed both little struggling and much floating, and the resultant line of rats was called "Swim Low-active" (SwLo). In parallel, we also chose for breeding those animals that showed the opposite – much struggling and little floating – and the resultant line of highly active rats was called "Swim High-active" (SwHi). Fig. 1 shows our swim test, as well as an illustration of both struggling and floating behavior.

The selective breeding and behavioral testing (in a variety of different behavioral tasks) of SwLo and SwHi rats is described in Weiss et al. [17]. As explained in Weiss et al. [17], selective breeding produced lines of rats that differed remarkably in their swim-test activity. In a 15-min swim test, SwHi rats now usually struggle for 200–500 s and float (or are immobile) for less than 20 s, whereas SwLo rats usually struggle for less than 10 s and float (or are immobile) for 600–800 s. Our laboratory presently houses the 45th generation of SwHi and SwLo rats.

Extensive testing of different antidepressant drugs has been done using the SwLo rat and is described in West and Weiss [18]. Summarizing briefly the results of antidepressant testing, the SwLo rat could be used to detect a wide variety of antidepressant drugs (i.e., by the antidepressant increasing their swim-test activity), but the SwLo rat did not appear to be any more responsive to SSRIs than normal, non-selected rats had been. Also, one of the antidepressant tested (amitriptyline) did not either increase struggling or decrease floating, so the conclusion reached was that the SwLo rat was not a type of animal that could readily detect all antidepressant treatments. However, the strong "positive" response of the SwLo rat to what have been described as "activating" antidepressants including tricyclics (Van Praag [19]), coupled with the low activity of these animals, suggested that the SwLo rat might be a model of atypical depression. It also can be noted that a positive response to antidepressants in the SwLo rat required chronic administration of antidepressant drugs (i.e., for two weeks), rather than their responding positively to antidepressant treatments delivered acutely (one-day treatment) as has been found when these drugs are given to normal animals, and so in this respect the SwLo rat is a better model of the human response to antidepressant drugs than is the normal rat. The SwHi rat, which shows very high activity in the swim test, not surprisingly proved to be not a very good test subject for screening of antidepressant drugs.

Mention should be made of additional studies which may bear on mechanisms that underlie the difference between SwHi and SwLo rats. West et al. [20,21] demonstrated that SwLo and SwHi rats have marked differences in the responsivity of forebrain dopamine systems that subserve motor activity. These studies show that SwLo rats, in comparison to SwHi rats, have a much-reduced response to

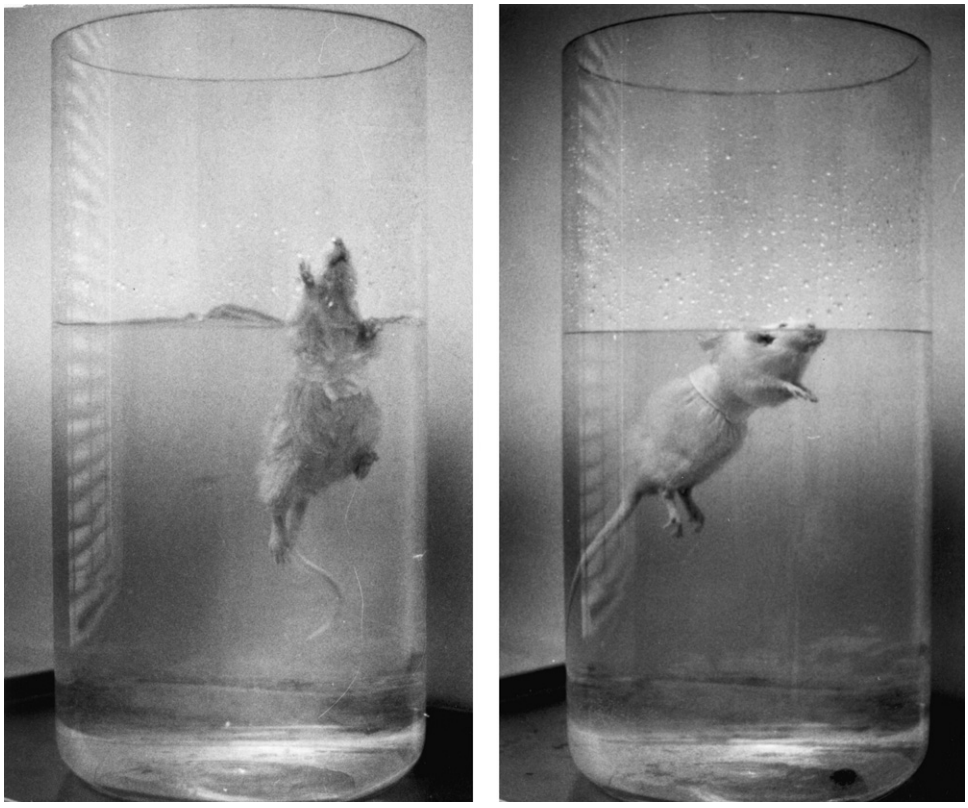

Fig. 1 – At left is shown an animal in the swim tank exhibiting struggling behavior, which is defined as vigorous motor activity with both front paws breaking the surface of the water, while at right is shown an animal exhibiting floating behavior (immobility), which is defined as the absence of any movement of limbs in the water. (Note that to prevent any sinking from occurring when rats cease active behavior, rats have air-filled bubbles attached to the back ("water wings") held in place by tape around mid-section which, as shown, does not restrict movement.) This test, and measurement of these behaviors, was used prominently in the selection and breeding of the lines of rats described in this paper. Swim Low-active (SwLo) rats were selected and bred for showing very little struggling and much floating in the swim test, while Swim High-active (SwHi) rats were selected and bred for showing much struggling and little floating in the swim test. Swim-test Susceptible (SUS) rats were selected and bred for showing greatly reduced struggling (and also show increased floating) after having been exposed to a stressful event, while Swim-test Resistant (RES) rats were selected and bred for showing essentially no decrease in struggling (and also show no increase in floating) after having been exposed to that stressful event.

drugs that stimulate dopaminergic receptors in forebrain dopaminergic regions such as the shell of the nucleus accumbens. Given the involvement of dopamine in these brain regions in motor activity, it can be hypothesized that the differential responsivity to dopamine seen in these regions plays a role in the mediating the differences in activity seen in the SwLo and SwHi lines.

2.2. *Swim-test susceptible (SUS) and Swim-test resistant (RES) rats*

The next lines of rats we attempted to develop were animals that would be either susceptible or resistant to stress. In particular, we had considerable interest in developing rats that were likely to show pronounced depression-like symptoms in response to stressful conditions (i.e., stress sensitive rats). As the fundamental stock for selective breeding, we used rats from an early generation of the Swim High-active rats (SwHi) because these rats would normally show considerable activity in the swim test. Animals were exposed to a stressful condition and then tested in the swim test. Based on swim-test scores, we then bred (a) animals that, following exposure to a stressful condition, showed large reductions in active behavior, particularly struggling behavior, in the swim test, and (b) animals that, following exposure to the same stressful condition, showed little or no reduction in their swim-test activity. Offspring of animals that showed large stress-induced reductions in swim-test activity gave rise to the ''Swim-test Susceptible'' (Susceptible or SUS) line of rats, while offspring of animals that showed little or no stress-induced reduction in swim-test activity gave rise to the ''Swim-test Resistant'' (Resistant or RES) line of rats. The derivation of these animals, neurochemical studies comparing SUS and RES rats of the fifth generation, and also electrophysiological and behavioral studies of these animals are described in Scott et al. [22]. The SUS and RES animals are indeed characterized by a number of physiological and electrophysiological differences within the brain as described in Scott et al. [22].

In the time since the original publication appeared, some notable developments have occurred. First, continued selective breeding of the SUS and RES lines (which are now each in the 40th generation) accentuated considerably the characteristics for which these animals were selected initially. Notably, when the selective breeding of these lines was begun, it was necessary to expose the rats to a highly stressful event (a 3.0 h session of electric shocks to the tail) in order to cause any rats to show a reduction in activity in the swim test (i.e., to produce any SUS rats for breeding). But beginning with the eighth generation of SUS rats, it was discovered that these rats had become sufficiently susceptible to stress that exposure to a session of tail shock was no longer necessary. In that generation (and thereafter), a marked reduction in swim-test activity was obtained by simply exposing SUS rats to a novel environment for 30 min in which a loud ''white noise'' sound (95 dB) is played. This mild stressor condition was used in carrying out the testing of antidepressant treatments described directly below, and continues to be the stressor condition used in selection and testing of SUS and RES rats up to the present time.

Second, we found that the SUS rat provides the basis for a screening technique that will detect effective antidepressant treatments. These studies were carried out with SUS rats of generations 12–17; by this point, SUS rats showed a reduction in swim-test struggling behavior of approximately 80% when exposed to the mild stressor described above (specifically, with no stressor applied SUS rats struggled for 80–90 s in a 15-min swim test, while they struggled for less than 20 s after being exposed to mild stressor condition described above). Effective antidepressant treatments (i.e., chronic administration of antidepressant drugs or electroconvulsive shock) were found to block this stress-induced reduction in swim-test activity seen in SUS rats (reported in West and Weiss [23]). Every class of antidepressant medication that was tested as well as electroconvulsive shock blocked the stress-induced reduction of swim test activity normally seen in SUS rats after they were exposed to the mild stressor. Additionally, a number of psychoactive drugs that often produce ''false positive'' responses in the swim test did not have this effect (i.e., chronic administration of these drugs did not block reduction of swim-test activity in SUS rats after they were exposed to the mild stressor). A summary of these results is shown in Fig. 2. Chronic administration of the antidepressant drugs was required to be effective, so that the responsivity of the SUS rat to antidepressant treatments in this procedure mimics the human response to antidepressants. The ability of this procedure to detect effective antidepressant treatments while not responding to a variety drugs that often produce ''false positives'' in the swim test is such that, based on the findings to date, this procedure is the most selective and specific preclinical screening technique for detecting effective antidepressant treatments that has been reported to date.

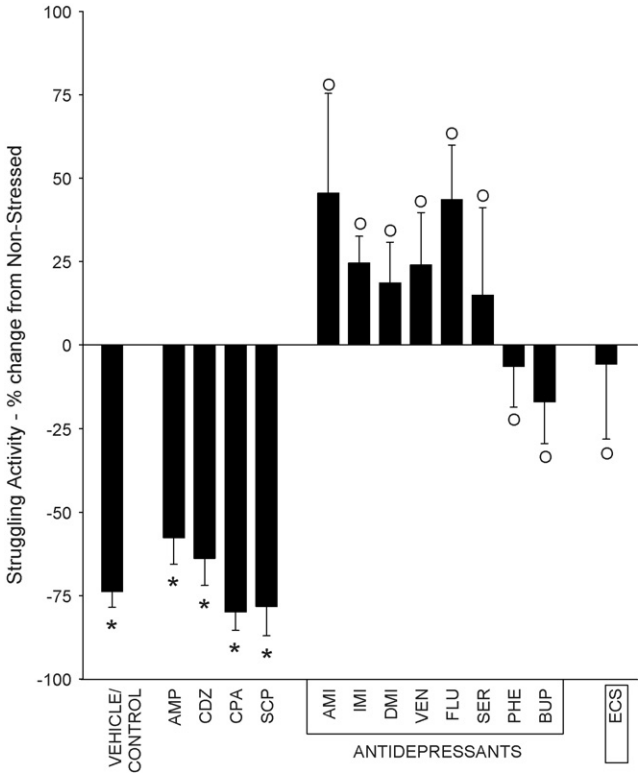

Fig. 2 – Percent change in swim-test struggling activity of Swim-test Susceptible (SUS) rats after exposure to a mild stressor when treated with various antidepressant or non-antidepressant drugs for 14 days or electroconvulsive shock. Means and standard errors are shown. "0" percent change represents the mean struggling of the non-stressed SUS rats that received each treatment, and the bar then shows the percent change from that level of struggling activity shown by the stressed SUS rats that received the treatment. Treatments (for 14 days via subcutaneously implanted minipump) were vehicle (VEHICLE/CONTROL), amphetamine [stimulant] (AMP), chlordiazepoxide [benzodiazapine tranquilizer] (CDZ), chlorpheniramine [antihistamine] (CPA), scopolamine [anticholinergic] (SCP), and the antidepressants amitriptyline (AMI), imipramine (IMI), desipramine (DMI), venlafaxine (VEN), fluoxetine (FLU), sertaline (SER), phenelzine (PHE), and bupropion (BUP). Animals were tested on the 14th day of drug treatment. For electroconvulsive shock (ECS), two shocks were given, and animals were tested on the sixth day after the second shock. (*) Differs significantly (at least $p < .05$) from "0" % change in struggling from non-stressed; (o) does not differ from "0" % change and differs significantly (at least $p < .05$) from percent of change seen in VEHICLE/CONTROL condition. These results show that all effective antidepressant treatments tested blocked the stress-induced decrease in struggling behavior that normally occurs in SUS rats, while none of the non-antidepressant treatments had this effect.

2.3. *Hyperactive (HYPER) rats*

Early in the selective breeding undertaking, while simply measuring baseline home-cage ambulatory activity of a large group of normal Sprague–Dawley rats, we observed that the male and female rats from a single litter were distinctly hyperactive during the dark period of the day (i.e., the rat's night) relative to the other rats being monitored. Brother–sister breeding of these rats was done, and their offspring have continued to show higher-than-normal spontaneous noctur-

nal ambulation in the home cage in succeeding generations. These animals have been designated as the ''Hyperactive'' (HYPER) line; they are now in the 38th generation.

An extremely interesting characteristic of the HYPER animals is their response to a stressor. Following exposure to a stressor, many young animals (i.e., 3–5 months of age) of this line show a period of extreme hyperactivity (i.e., nocturnal ambulatory activity counts often exceeding 1000 counts per hour) that begins 2–5 days after the stressor has been applied and then lasts for 3–6 days. Following this period of extreme hyperactivity, their activity returns to its pre-stress baseline. Almost needless to say, randomly-bred ''normal'' animals do not show this; following exposure to a stressor, normal animals may show a brief depression in their behavioral activity but in a day or two they simply return to their pre-stress level without appearance of any hyperactivity. If the outburst of extreme hyperactivity shown by HYPER rats is related to mania, the HYPER rat would represent the first endogenous model of mania (i.e., hyperactivity [or mania] that is not produced by a surgical manipulation or by drugs) that has been reported to date.

Another very interesting characteristic of HYPER rats is that they can also show profound behavioral depression as well. When older male HYPER rats (i.e., 10–14 months of age) are exposed to a single 3-h stress session (uncontrollable shock), they show a profound reduction in ambulatory activity in the home cage that lasts 3–5 weeks (i.e., 21–35 days), accompanied by a reduction in food and water intake that lasts for 2–3 weeks. Normal male Sprague–Dawley rats of similar age (i.e., non-hyperactive rats) do not show this; normal male Sprague–Dawley rats of this age respond to being exposed to a single 3-h stress session of uncontrollable shock with a decrease in activity and food/water consumption that does not last beyond 5–7 days. The long-lasting depression of motor activity in the home cage, as well as reduction in food/water intake, shown by HYPER rats after they have been exposed to an acute environmental event (i.e., a single 3-h stress session) represents a potential advance in our ability to model depression in the rodent. This not only mimics the clinical picture of a ''precipitating incident'' giving rise to depression, but also meets the previously-elusive DSM depression criterion for symptom persistence in the rodent in that symptoms last ''for at least two weeks'' (DSM-IV^{TM} [24]). Additionally, the symptoms are sufficiently long-lasting in this model to permit testing of antidepressant treatments, particularly antidepressant drugs, that generally require several days, or as long as two weeks, in order for these treatments to be effective; the depressive condition can be induced, drug then given, and recovery from the depressive condition then monitored to determine effectiveness of a treatment requiring chronic administration. An example of this is shown in Fig. 3. In this particular case, testing was done of an experimental antidepressant treatment – blocking of galanin receptors in the ventral tegmentum (VTA), which was done by microinfusion into VTA of the receptor blocker Galantide (Weiss et al. [25]). Note in Fig. 3 that the ''control'' animals (i.e., Vehicle-treated HYPER rats) took nearly 30 days before their nocturnal ambulation level returned to their ''baseline'' level after being exposed to a single 3-h stress session (given just prior to shock night [Shock Nt.]). This illustrates the long-lasting depression of motor activity that can

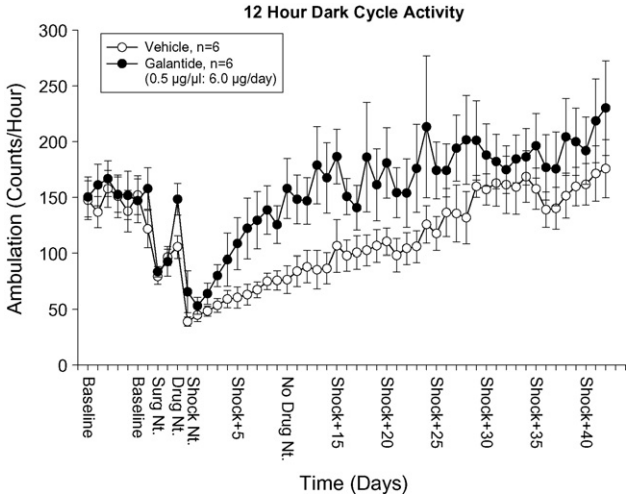

Fig. 3 – In HYPER rats, the effect of continuous bilateral infusion (by minipump; 14-day infusion capacity) of the galanin receptor antagonist Galantide (6.0 μg per day per cannula) or no drug (vehicle) into the ventral tegmentum (VTA) on recovery from prolonged depression of motor activity in the home cage that follows exposure of HYPER rats to one session of uncontrollable shock. As explained in the text, one-year old male HYPER rats show prolonged depression of motor activity following exposure to a single 3-h session of uncontrollable tail shocks; examination of the no-drug (vehicle) animals shows the slow return to baseline level of home-cage nocturnal activity after the shock session was given. Infusion of Galantide into VTA hastened the recovery of home cage motor activity following the shock session. Measure shown is hourly ambulatory activity during the 12-h period of darkness of each day (when rats are active); mean and standard error for each group is shown. Key to labels on horizontal axis – "Baseline"-"Baseline": seven days of no treatment used to establish baseline level of activity; "Surg Nt.": night following rats undergoing stereotaxic surgery to implant bilateral cannulae into VTA and attachment of minipumps for vehicle or drug infusion into VTA; "Drug Nt.": night following clearance of CSF from end of cannulae and beginning of vehicle or drug (Galantide) infusion into VTA; "Shock Nt.": night following the 3-h shock session having been given; "S + 1" "S + 5", etc.: night number after shock session had been given (e.g., "S + 1" = shock night plus one); "No Drug Nt.": approximate point at which minipump is exhausted and ceases to deliver drug or vehicle.

be seen in older male HYPER rats after exposure to a single, highly stressful event. It can also be noted in Fig. 3 that Galantide infusion into VTA accelerated recovery of depressed motor activity, thereby suggesting that galanin antagonists may possess antidepressant qualities.

Thus, the HYPER rat can show long-lasting depression-like symptoms as well as manic-like outbursts. The HYPER rat may therefore possess attributes of bipolar disorder; if so, this would be one of the few models, and perhaps the only homologous model discovered to date, of this disorder.

2.4. *Monitor resistant (MonRES) rats*

At the very outset of the selective breeding effort described here, we attempted to selectively breed for animals that would show reduced ambulatory activity in the home cage after being exposed to a stress session consisting of uncontrollable electric shocks. Male and female rats were exposed to this stressor and their ambulatory activity was measured thereafter; those male and female rats showing large reductions in home-cage activity were mated. The effort ended in failure – rats that showed large decreases in home cage ambulation after being exposed to a stressful event eventually stopped producing offspring. (This could be viewed as adaptive evolutionary development by which extremely stress-sensitive animals are eliminated from the population because they cease to produce offspring.) In any event, in parallel with this effort we also selectively bred males and females that showed the converse – that is, rats that were highly resistant to showing any stress-induced reduction in ambulatory activity. Since ambulatory activity was recorded in the monitor room, these were referred to as ''Monitor Resistant'' (MonRES) rats. This line has been perpetuated. It is based on selective breeding over the initial five generations, and has been maintained by breeding rats of this line without continued selection thereafter.

3. Consumption of alcohol and drugs of abuse by selectively-bred rat lines

Based on preliminary neurochemical findings that pointed to the presence of marked differences in brain monoamine metabolism of different lines of selectively-bred rats, we were motivated to assess whether different lines would consume alcohol and other drugs of abuse. The willingness of selectively-bred rat lines to consume alcohol, amphetamine, and cocaine has been examined. The propensity to consume alcohol has been tested more extensively than the other drugs, and with regard to amphetamine and cocaine consumption not all lines have been assessed. The findings are described below.

3.1. *Alcohol consumption*

Dr. Charles West became intrigued by the possibility that the Susceptible (SUS) rat might show the propensity to consume alcohol; this was based on preliminary results we obtained indicating that the level of serotonin in the brain of SUS rats was lower than normal. He therefore led the work assessing alcohol consumption in SUS and RES rats, as well as in SwLo and SwHi rats; the findings of this investigation are described in West and Weiss [26]. When rats in the home cage were continuously provided with both normal drinking water and a 10% ethanol solution, SUS rats consumed considerable quantities of the 10% ethanol solution; in fact, they drank more of this solution than they did the plain water. In contrast, normal rats as well as other rat lines tested (i.e., RES, SwLo, and SwHi rats) showed, as is normally found, a marked aversion to the 10% ethanol solution (see Figs. 4 and 5). The propensity for SUS rats to consume alcohol was confirmed and expanded in experiments where intake of different concentrations of alcohol was assessed. SUS rats also showed the tendency to consume more of palatable substances such as saccharine and sucrose solutions. Interestingly, SUS rats also showed more *aversion* to bitter tasting quinine solutions than did normal rats, thus showing that their preference for alcohol was not due to their being less sensitive than normal to bitter taste aspects of alcohol. Overall, the proclivity of SUS rats to consume alcohol was as strong as that seen in various rat lines that were specifically bred for willingness to drink alcohol; thus, the SUS rat, which was selected and bred for being susceptible to stress with no intended relationship to alcohol consumption, shows a tendency to consume alcohol that is as strong as that of rats specifically bred for alcohol consumption.

3.2. *Amphetamine intake*

Based on findings that SwLo and SwHi rats differ markedly in their response to drugs that stimulate dopaminergic receptors in the forebrain dopaminergic regions such as the nucleus accumbens, Dr. West led our group in assessing whether these lines would differ in their willingness to consume amphetamine. When solutions containing amphe-

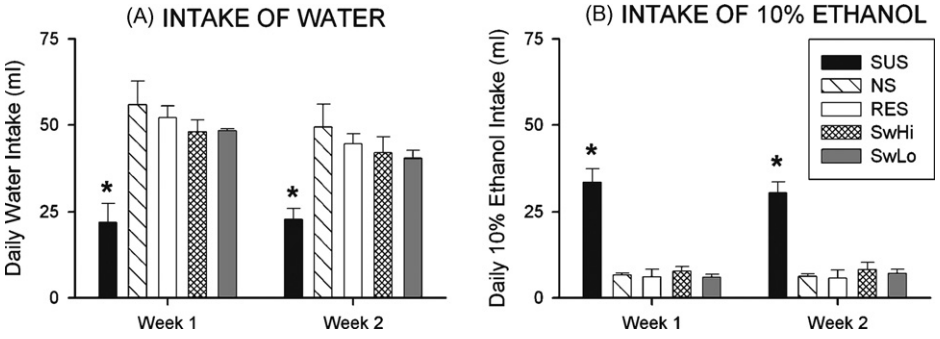

Fig. 4 – Daily intake of water (A) and 10% ethanol solution (B) by different lines of rats during two weeks of testing. Water and the 10% ethanol solution were continuously available throughout the two-week period. Lines of rats were Swim-test Susceptible (SUS), non-selected (i.e., normal) rats (NS), Swim-test Resistant (RES), Swim High-active (SwHi) and Swim Low-active (SwLo). Means and standard errors are shown. (*) Differs significantly (at least $p < .05$) from each of the other lines.

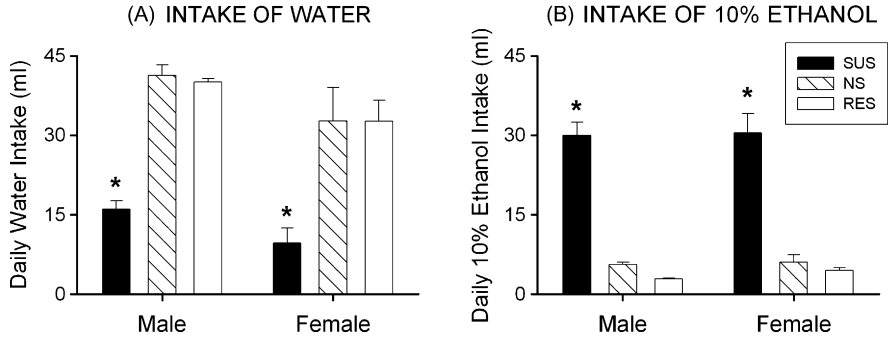

Fig. 5 – Daily intake of water (A) and 10% ethanol solution (B) by male and female rats from different lines during 10 days of testing. All other details, including designations of rat lines, can be found in the legend for Fig. 4.

tamine were offered to SwLo, SwHi, and non-selected (i.e., normal) rats along with plain drinking water in the home cage, none of the rats would ingest appreciable amounts of the amphetamine solution. Therefore, we adopted the procedure of restricting the animals' time of water intake, giving them water for 2 h per day in the early morning just after the conclusion of their "dark" phase of the day. After they had adapted to this schedule, the 2-h period was divided; the rats were first given a water bottle containing an amphetamine solution for 1 h, followed by plain drinking water for the second hour. Three amphetamine concentrations were utilized (0.0125, 0.025, and 0.05 mg/ml), each concentration given daily for one week. Fig. 6 shows the findings when 0.05 mg/ml was tested, which produced the largest difference in amphetamine intake. SwLo rats ingested in the most amphetamine in this test situation, non-selected (i.e., normal) rats took in somewhat less than the SwLos, and SwHi rats took in the least.

Next, we assessed whether these lines would take in sufficient amphetamine to affect their behavior. In this aspect of the study, animals were, as before, given access to plain drinking water for 1 h per day, but they also had access to the 0.05 mg/ml amphetamine solution in a second bottle for 24 h per day. A "control" (or no drug) condition consisted on giving them plain drinking water for 24 h per day. Each of these conditions was continued for one week, and ambulatory activity was measured throughout. In this study, similar differences in amphetamine consumption occurred as are shown in Fig. 6 – SwLo rats consumed the most amphetamine (mean and standard error [S.E.] = 0.94 ± 0.12 mg/kg), non-selected (NS) rats consumed somewhat less amphetamine (0.78 ± 0.1 mg/kg), and SwHi rats took in clearly the least amount of amphetamine (0.34 ± 0.04 mg/kg) (these data not shown in a figure). Interestingly, consumption of the amphetamine was sufficient to produce increases in motor activity in the home cage. The increase in activity while having access to amphetamine was most marked in SwLo and non-selected animals, whose nocturnal activity (dark phase activity) rose from 60–70 ambulatory counts per hour in the non-drug condition to 90–110 ambulatory counts per hour on the drug-available days (for all of these groups [see Fig. 6 for groups], the increase over their "no drug" activity level was statistically significant [at least $p < .01$]). For the SwHi rats, the increase in activity was smaller, moving up from a higher "no-drug" baseline level than was seen in the other groups – approximately 95 ambulatory counts per hour – to 108–115 counts per hour during the drug-available period; nevertheless, this smaller increase in activity was statistically significant for one of the SwHi groups (SwHi-b). In the other SwHi group, the increase did not reach statistical significance (i.e., $p = .10$) because, while six of the rats were clearly affected by the amphetamine (mean increase = 39

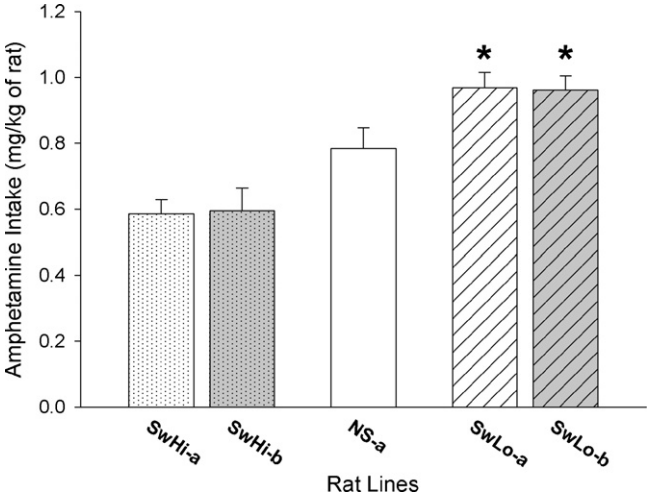

Fig. 6 – Consumption of an amphetamine solution (0.05 mg/ml) by rats from different lines. Rat lines were Swim High-active (SwHi) and Swim Low-active (SwLo), with rats from the original lines developed by selective breeding (SwHi-a and SwLo-a) and also from parallel lines that were separated from the original lines at generation 13 and selectively-bred separately thereafter (SwHi-b and SwLo-b). Also included were non-selected (normal) rats bred in parallel with the original SwHi and SwLo lines (NS-a). Means and standard errors are shown; consumption is expressed as mg/kg body weight of the rats. (*) SwLo-a or SwLo-b differs significantly ($p < .001$) from SwHi-a or SwHi-b, which was determined by carrying out comparisons of individual groups following a one-way analysis of variance for the five groups shown here; that analysis yielded a significant overall effect of group ($F = 12.7$, $p < .001$). Differences between the NS-a line compared with the SwHi lines or the SwLo lines approached, but did not reach, statistical significance (i.e., $p < .10$).

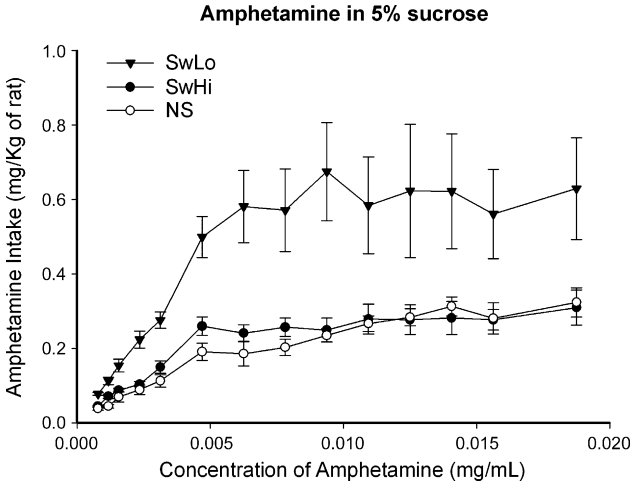

Fig. 7 – Consumption of amphetamine in a 5% sucrose solution by rats from different lines. Rat lines were Swim High-active and Swim Low-active rats from the original lines developed by selective breeding (SwHi and SwLo) as well as non-selected (normal) rats bred in parallel with the SwHi and SwLo rats (NS). Rats were given access to the sucrose solution containing amphetamine for 3 h on two occasions each week, with the concentration of amphetamine in the solution increased in each successive week (see text for details). Means and standard errors are shown; consumption is expressed as mg/kg body weight of the rats. Statistical analysis: a repeated measures analysis of variance revealed a significant effect of dose of amphetamine ($F = 44.5$, $p < .001$), rat line ($F = 9.9$, $p < .003$) and a significant rat line × dose interaction ($F = 4.6$, $p > .001$). Similar analyses of variance done to compare individual groups yielded a significant rat line effect for SwLo vs. NS ($F = 11.5$, $p < .01$), SwLo vs. SwHi ($F = 9.6$, $p < .01$), and no significant difference for SwHi vs. NS (0.4, $p = .52$).

ambulatory counts per hour in these rats), the other six rats, which took in little amphetamine as did the all SwHi rats, were essentially unaffected.

Finally, we endeavored to see if these lines would voluntarily take in amphetamine if the drug were placed into a palatable solution. Therefore, amphetamine was dissolved in a 5% sucrose solution. The animals, which lived in their home cages with water available at all times, were given this solution for 3 h on two occasions each week, with the position of the amphetamine solution and the water reversed on the two presentations. The concentration of amphetamine was progressively increased, with a higher concentration in the solution each week. The results are shown in Fig. 7. Again under these conditions, SwLo rats took in the most amphetamine, with non-selected (normal) and SwHi rats not differing from each other in their intake.

3.3. *Cocaine intake*

To assess whether SwLo rats would take in larger quantities of cocaine than normal rats, cocaine intake was compared in SwLo and non-selected rats, and intake by Susceptible (SUS) rats was measured as well. In the home cage, the animals were presented with a cocaine solution in addition to the water bottle two times each week; the two-bottle choice was left in place for 24 h on each occasion that it was offered. The position of the water bottle and cocaine solution was alternated in each week. The concentration of cocaine was 0.02% throughout; the experiment was continued for six weeks. The results are shown in Fig. 8. While the SwLo and non-selected animals began by taking in the same amount of cocaine (see first week), the non-selected rats soon began to avoid the cocaine solution and by week 3 were taking in very little cocaine. SwLo rats, in contrast to this, increased their intake of the cocaine solution slightly in the second week, and maintained that level of intake throughout the experiment. SUS rats in the first week ingested slightly less of the cocaine solution than both of the other two groups, but, as can be seen in Fig. 8, SUS rats maintained their intake over the six week duration of the study, so that ultimately the SUS rats consumed less cocaine than did SwLo rats but considerably more than non-selected rats. An interesting feature of the cocaine intake of all lines, but particularly of the SwLo and SUS rats that showed the tendency to consume significant amounts of cocaine, was that their intake reached a particular

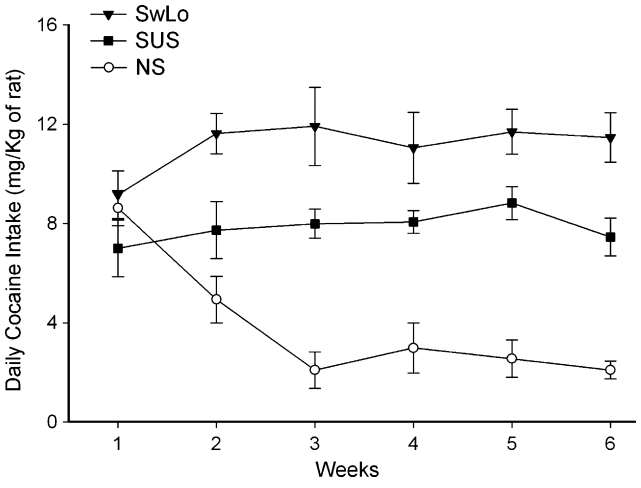

Fig. 8 – Consumption of cocaine in drinking water by rats from different lines. Rat lines were Swim Low-active (SwLo), Swim-test Susceptible (SUS), and non-selected (normal) rats (NS). Cocaine concentration in drinking water was 0.2 mg/ml; rats had access to the cocaine solution for two 24-h periods during each week across six weeks; cocaine consumption is the amount consumed on those days when it was available. Means and standard errors are shown; consumption is expressed as mg/kg body weight of the rats. Statistical analysis: a repeated measures analysis of variance revealed a significant effect of rat line ($F = 57.3$, $p < .001$) and a rat line x week interaction ($F = 13.0$, $p < .001$). Comparisons between individual groups at each week showed SwLo differed significantly (at least $p < .05$) from NS in each of weeks 2–6, SUS differed significantly from NS in each of weeks 3–6, and SwLo differed significantly from SUS in weeks 2, 3, and 6.

dose level and the animals then maintained that dose of intake. Such results point to each rat line having a particular sensitivity, or responsivity, to the drug, which then limits drug intake to that asymptote.

3.4. Summary

The findings related to consumption of substances of abuse – alcohol, amphetamine, and cocaine – by various lines that were selectively bred for behavioral characteristics indicate that these lines (a) show propensities to consume such substances, and (b) differ from one another, as well as from normal, non-selected rats, in their propensity for consumption of these substances. First, the SUS rats are unique amongst the lines tested in showing a pronounced proclivity to ingest alcohol. The propensity of SUS rats to consume alcohol is as strong as that of rat lines especially bred in other laboratories for alcohol intake [27–29]. Second, SwLo rats show a greater propensity to consume amphetamine and cocaine than do normal rats, and a considerably greater propensity to do so than do SwHi rats. Of considerable interest, all lines tested apparently consumed both amphetamine and cocaine up to a ceiling level (for that rat line) and then maintained consumption at that level thereafter. This observation is consistent with idea that different rats (i.e., different rat lines) have different sensitivities to drugs, and that their willingness to consume the drug is limited by this sensitivity. A direct extrapolation from this is that individuals having a low sensitivity to a drug (for example, SwLo rats to amphetamine) are able to take in large amounts of that substance, and therefore may be prone to abuse it, whereas individuals that are highly sensitive to a drug (for example, SwHi rats to amphetamine) are unwilling to take in large amounts of that substance, and therefore are less prone to abuse it.

4. Review of monoaminergic measurements made previously in other selectively-bred lines of rats

Before presenting the findings from measurement of monoamines in the brain of the selectively-bred lines of rats described previously, we will briefly review what has been reported with respect to brain monoamines in other lines of selectively-bred rats. Given the suspected importance of brain monoamines for affective disorders and addictive behavior, brain monoamines have been studied in other selectively-bred lines of rats as well as the ones described in this paper.

A larger number of selectively-bred rat lines have been developed in the area of alcohol abuse than in any other research area relevant to addiction and affective disorders. Rats (and other rodents) will not normally consume alcohol in appreciable quantity; in fact, the normal rodent will avoid its consumption. In order to use rodents to study alcohol abuse, investigators therefore have selected both male and female rats that show the propensity to consume alcohol and have bred these animals in order to generate lines of rats that will consume large amounts of alcohol. These strains include the P and NP rats (alcohol-preferring [P] and non-preferring [NP]) which were selectively bred at the University of Indiana using outbred Wistar rats as the foundation stock, the Sandinian P and NP rats (also alcohol-preferring and non-preferring, designated sP and sNP) which were also selectively bred from Wistar rats but derived in Cagliari, Italy, the HAD and LAD rats (high alcohol drinking [HAD] and low alcohol drinking [LAD]) which were selectively bred using the N:NIH rat stock (supposedly embodying considerable variability in alleles across the entire rat genome) rather than outbred Wistars, and the Myers HEP rats (Myers high ethanol preferring [mHEP]) which were bred by crossing male P rats with female Sprague–Dawley rats that showed ethanol preference. Levels (i.e., concentrations) of monoamines and various metabolites have been measured in brain regions of the rats lines listed in the previous sentence.

The two monoamines discussed most prominently in regard to alcohol-preferring and non-preferring rat lines are dopamine (DA) and serotonin (5-HT). Relevant to the lines of alcohol-ingesting rats described above, there are several reports that DA, and indicants of DA release, are reduced within forebrain dopaminergic regions (i.e., the mesocorticolimbic dopaminergic system and the striatum) of rats that ingest appreciable quantities of alcohol. Murphy et al. [30] found the P rats, compared to NP rats, had lower levels of DA and the dopaminergic metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the nucleus accumbens (NAC), as well as lower levels of HVA in the striatum (STR). Gongwer et al. [31] reported similar findings in HAD vs. LAD rats, and McBride et al. [32] also found lower DA in the NAC of both P and HAD rats relative to the appropriate control, non-alcohol ingesting rats. Lucas and McMillen [33], while finding a higher level of DA in the prefrontal cortex (PFC) of the Myers HEP rat relative to controls, found at the same time that the Myers HEP rat had lower levels of DOPAC and HVA in that brain region as well as lower DOPAC in the NAC. However, it needs to be noted that not all studies are consistent with the view that animals prone to ingest alcohol show lower activation and release of DA in forebrain dopaminergic systems. Morzorati [34] and Morzorati and Marunde [35] conducted electrophysiological recordings of the firing of ventral tegmentum dopaminergic cells (VTA-DA cells), which constitute the dopaminergic cell bodies whose axons project to the mesocorticolimbic terminal regions (i.e., NAC, PFC). They reported that burst firing of these VTA-DA cells, which releases more DA than occurs at basal firing rate, was higher in P than NP rats. Consistent with this, Fadda et al. [36] observed that, in sP as compared to sNP rats, there was no difference in the endogenous level of DA in the STR, but administration of ethanol to these rats reduced striatal DA in sP rats and caused a larger increase in striatal DOPAC and HVA in the sP rats compared to sNP rats.

With respect to serotonin (5-HT), there are several reports of the alcohol-ingesting lines of rats showing, in various brain regions, reduced 5-HT levels together with reduced serotonin release as indicated by reduced levels of the serotonergic metabolite 5-HIAA. Murphy et al. [30] reported lower levels of 5-HT in NAC, STR, and PFC as well as lower levels of 5-HIAA in PFC in P rats compared to NP rats. Gongwer et al. [31] found lower levels of 5-HT and 5-HIAA in several brain regions including STR and PFC in HAD rats compared to LAD rats. McBride et al. [32] confirmed findings of lower 5-HT levels and

5-HIAA in both P and HAD rats. Additionally, Lucas and McMillan [33] observed that levels of 5-HT and 5-HIAA in the NAC were lower in female mHEP rats than in the ethanol non-preferring controls.

Summarizing the findings in alcohol-ingesting lines of rats described above, differences in DA and 5-HT levels and also release as indicated by metabolite concentration have been seen in the brains of the these rat lines. The most consistent finding is reduced 5-HT and 5-HT release in the forebrain, but there are also a number of reports of reduced DA and DA release, particularly in forebrain regions including the basal ganglia and mesocorticolimbic dopamine system.

In comparison to selectively-bred lines of rats intended for studies of alcohol abuse, fewer lines have been developed previously for other purposes such as studies of affective disorders and emotionality. However, some lines of rats have been generated for these purposes, and a limited number of studies of monoamines in the brain also have been carried out in these lines. These will now be described.

Rat lines have been bred for different levels of emotionality. Amongst the first selectively bred rat lines to be developed were the Maudsley Reactive Rat and the Maudsley Non-reactive rat. Originally selectively bred by P.L. Broadhurst at the Maudsley Psychiatric Institute in London (e.g., Broadhurst [37,38]), these lines of rats were intended to differ in emotionality, which was assessed by testing rats in the open field test and quantified by how much the rats defecated in this test. The reactive rat (presumed to be highly emotional) was selectively bred on the basis of showing high defecation in the open field, and the non-reactive rat was bred on the basis of showing low defecation in this test. In an early report, Sudak and Mass [39] reported that male rats of the Maudsley Reactive line (MR) had higher 5-HT levels throughout the brain than did Maudsley Non-Reactive rats (MNR). Slater et al. [40] and Liang and Blizzard [41] measured brain norepinephrine (NE), and reported that NE level was higher in the brainstem and telencephalon of MR rats than in the non-reactive rats (they used MNRA rats, a subline of the MNR rat). Interestingly, they also found that the non-reactive rats (MRNA) conversely showed higher levels of NE in the hypothalamus than did the MR rats. Such findings suggest that the noradrenergic locus coeruleus (LC) neurons in the brainstem that project to the telencephalon are more active (and therefore synthesize more NE, resulting in higher levels of NE in throughout the telencepahalon) in the reactive rats than in non-reactive rats, with this difference being possibly due to the non-reactive rats having a higher inhibitory influence on LC firing exerted through NE in the ventral bundle system which projects not only to LC but also to hypothalamus.

Landgraf and colleagues selectively bred Wistar rats for differences in emotionality by selecting rats based on their performance on the elevated plus maze (EPM). This maze, which is in the form of a ''+'' elevated above the floor, has open arms and closed arms; highly emotional/anxious rats avoid the open arms of this maze, and therefore spend much time on the closed arms and little time on the open arms. In these rat lines that were bred for high anxious behavior (HAB) and low anxious behavior (LAB), Langraf and colleagues have concentrated on exploring differences in pituitary-adrenal responsivity and central determinants of this response such as vasopressin and corticotropin releasing hormone (e.g., Langraf et al. [42]). However, they have also examined some aspects of brain monoamine function, having assessed hippocampal serotonin by microdialysis (Keck et al. [43]). Under ''resting'' conditions (i.e. non-stress conditions), the amount of 5-HT found in extracellular fluid of the hippocampus did not differ in HAB as compared to LAB rats, but exposure to the EPM increased extracellular 5-HT in LAB rats while having no effect in HAB rats. The authors concluded that 5-HT release was reduced in the hippocampus of HAB rats.

In another comparison of rat lines thought to differ in emotionality, Kulikov et al. [44] compared aspects of serotonergic function in two inbred lines of rats that also differed in ''anxiety'' as assessed on the EPM. These investigators compared the Lewis rat (LEW) with the spontaneously hypertensive rat (SHR), with the LEW rat having been found to be considerably more anxious than the SHR as indicated by performance on the EPM. These investigators reported that in PFC, STR, hippocampus, and midbrain, neither 5-HT synthesis nor radioligand binding to several key 5-HT receptors differed in these two lines.

Summarizing the findings in rat lines bred for, or manifesting, high emotionality as opposed to low emotionality, the findings show no clear pattern of relating this difference to monoamines. 5-HT was examined most often in these lines. In high emotional rats, the brain level of 5-HT was elevated in one line (MR rats), 5-HT release was reduced in another line (HAB), and no difference between high and low emotional rats was found in another comparison (LEW vs. SHR). In the one instance where NE was measured (Maudsley lines), NE was elevated in the projection regions of the dorsal bundle-locus coeruleus system of the high emotional rats line relative to the low emotional line, while NE was also lower in the hypothalamus of the high emotional animals, which is an important projection field of the NE ventral bundle whose cell bodies lie posterior and lateral to the locus coeruleus.

Three selectively-bred rat lines that are potentially relevant to the study of depression have had aspects of brain monoamines assessed. First, Fritz Henn, Emeline Edwards, and colleagues bred rats that were selected for showing poor performance in an ''escape from electric shock'' task after having been exposed to uncontrollable electric shocks previously; this deficit had been called ''learned helplessness'' and therefore the rats were referred to as being a line of ''helpless'' rats. The behavioral deficit labeled ''learned helplessness'' has been hypothesized to be representative of (i.e., a model for) depression (Seligman [45]). In the poor escape-performing rats (called ''helpless'' rats), Martin et al. [46] reported that these animals had higher levels of 5-HT in the hippocampus. Edwards et al. [47] confirmed the same finding, and also reported that K^+ stimulated release of 5-HT from hippocampal slices in vitro was elevated in these rats. No differences in release of NE, DA, or acetylcholine (Ach) from the slices were observed.

Second, another line of rats thought to be relevant for studies of depression is the Flinders sensitive rat (FSL). The FSL rat was selectively bred based on high responsiveness to the anticholinesterase drug diisopropylfluorophosphate (DFP), as measured by changes in body temperature, drinking, and body weight, in order to generate a line of rats that are highly

sensitive to acetylcholine (Overstreet et al. [48]). The Flinders Resistant rat (FRL) showing the opposite tendency was also developed in parallel. The FSL rat has been proposed to be a genetic animal model of depression, and Overstreet [49] has reviewed the supporting evidence. Zangen et al. [50] reported that the FSL rat had higher levels of 5-HT and its metabolite 5-HIAA in NAC, PFC, hippocampus, and hypothalamus than did normal (i.e., randomly-bred) Sprague–Dawley rats. Zangen et al. [51] then reported that NE levels of FSL rats were higher in NAC, PFC, hippocampus, and median raphe than were found in normal Sprague–Dawley rats. Additionally, this report indicated that FSL rats had higher levels of DA in NAC and STR, as well as higher levels of DOPAC and HVA in these brain regions.

Third, Overstreet, Janowsky and colleagues also bred rats selectively for differences in their response to 5-HT_{1A} receptor stimulation (Overstreet et al. [52]); differences in this receptor have been associated with depression as well as with alcoholism and anxiety (Knapp et al. [53]). Rats were selected from the N:NIH stock thought to embody variability in alleles across the entire rat genome, and were selectively bred based on the rats (in a foundation stock population of 30 N:NIH rats) showing either the largest or smallest hypothermic responses rats to an injection of the 5-HT_{1A} agonist 8-OH-DPAT. In addition to the high sensitivity line that showed the largest hypothermic responses (HDS) and the low sensitivity line that showed the lowest responses (LDS), a randomly-bred control line from the foundation stock was maintained as well (RDS). In four generations of selective breeding, separation of the lines with respect to the hypothermic response was obtained. Behavioral testing of the resultant rat lines (i.e., HDS, LDS, RDS) showed no consistent differences in the open field test, on the elevated plus maze (EPM), or in preference for alcohol, so that these rat lines, despite significant differences in the functional responsiveness of their 5-HT_{1A} receptors, do not appear relevant to the clinical problems of anxiety or alcoholism. But in the swim test, which as explained earlier is used to screen for effective antidepressant drugs, the HDS rats were more immobile than were the LDS and RDS rats, suggesting that the lines might be relevant for the study of depression (Overstreet et al. [54]). However, it is important to note that by the seventh and eighth generation both the very high sensitivity line (HDS) and the very low sensitivity line (LDS) lines showed significantly more immobility in the swim test than did the RDS randomly-bred ''control'' rats, so it is also evident that ''depression-like behavior'' (i.e., immobility in the swim test) does not correlate well with 5-HT_{1A} receptor responsivity. With respect to assessment of monoamines, Gonzales et al. [55] reported microdialysis results in the PFC and hippocampus of these lines. In both brain regions, HDS and LDS rats had lower basal 5-HT release (lower extracellular 5-HT) than did the RDS control animals, so that this measure paralleled what was seen in the swim test with respect to immobility. The HDS line showed a larger response to fenfluramine than both other lines but this was seen only in the hippocampus.

Summarizing the findings in rat lines bred for relevance to depression, across the various lines that have been developed the findings show no clear pattern. The monoamine receiving the most attention again has been 5-HT. Rats bred for showing ''helplessness'' (i.e., poor escape behavior following exposure to a stressor) showed elevated 5-HT release from hippocampal brain slices in vitro, and the FSL rat, hypothesized to be a model for study of depression, showed elevated 5-HT levels in several brain regions. However, in rats bred for either high or low 5-HT_{1A} receptor sensitivity (high 5-HT_{1A} receptor sensitivity was thought to be related to depression), both lines both showed more immobility in the swim test than did randomly-bred controls, and these lines also manifested lower 5-HT release in PFC and hippocampus (measured in vivo by microdialysis) than did the randomly-bred controls. Thus, in rat lines bred for differences in 5-HT1A receptor sensitivity, low 5-HT release, rather than high 5-HT release, accompanied the ''depression-like'' response. Considering other monoamines, when NE and DA were measured in the FSL rats, NE in the NAC, PFC, hippocampus, and median raphe was higher in FSL rats than in randomly-bred Sprague–Dawley rats, as was DA and DOPAC in NAC and striatum; however, the consistency of these differences with respect to rat models of depression cannot be judged as these measures were not made for the other lines.

The final group of rats that will be addressed in this review are lines that were selectively bred for showing fast vs. slow development of amygdala kindled seizures. These were originally developed from a parent population of Wistar and Long-Evans hooded rats, selectively-bred for 11 generations beginning in the 1970s (Racine et al. [56]), and then maintained without selection for 30 generations prior to the measures reported here. McIntyre et al. [57] reported on measurement of brain monoamine (NE, DA, and 5-HT) and metabolite level in many brain regions, similar to the type of data that will be presented here for our selectively bred rat lines. Additionally, McIntyre et al. subjected the animals to two different stressors (restraint and exposure to a ferret) and reported changes in monoamines and metabolites produced by these stimuli. These investigators reported that the Fast and Slow rats showed some marked behavioral differences, with Slow rats appearing to be more anxious than Fast rats by exhibiting fewer open arm entries on the EPM, greater freezing in an avoidance task, and slower acquisition of an active avoidance task. Also, Slow rats readily adopted a passive response when placed in restraint while Fast rats struggled in this situation. Despite these marked behavioral differences, McIntyre et al. reported that in almost all brain regions monoamine levels were similar and stressor-induced changes were also similar. One exception to this, however, was seen in the effect of stress on the level of NE in the locus coeruleus – in Fast rats, exposure to either stressor caused NE levels in the locus coeruleus to fall over 25% from the non-stress (control) level, whereas these same stressors had hardly any effect on NE level in this brain region in the Slow rats. Anisman et al. [58] reported further results with respect to monoamines in these rats. A prominent finding was that, again in the locus coeruleus, presentation of a stimulus (either a blinking light or a blinking light that had been paired with shock) produced a large increase in the noradrenergic metabolite 3-methoxy-4 hydroxyphenylglycol (MHPG) of Fast rats, while presentation of these stimuli had no effect on MHPG in this brain region of Slow rats. In Fast rats, MHPG elevations in response to stimulus presentation were often more pronounced than in Slow rats in other brain

regions as well. These results suggest that locus coeruleus neurons, and consequently the dorsal bundle noradrenergic system, are more subject to activation in Fast rats than in Slow rats. Finally, the investigators noted that the basal level (i.e., non-stress level) of the metabolite DOPAC, which is mostly derived from dopaminergic neurons, was higher in the PFC of Fast rats than in Slow rats, and suggested that the impulsive behavior of Fast rats might be related to excessive dopaminergic function in the PFC.

5. Differences in brain monoamine content and metabolism in swim high and low active, susceptible and resistant, and hyperactive rat lines

Having reviewed findings related to brain monoamines in other selectively bred lines of rats, this paper now presents the findings from measurement of brain monoamines and their metabolites in the rat lines described earlier in this report. As pointed out in regard to assessment of drug intake by these selectively-bred rat lines, such measurements were made in response to preliminary data suggesting that the rat lines we have generated differ in monoamine content and metabolism in various brain regions. Various hypotheses have stipulated that such neurochemical differences are important for regulating drug use. In this section, we present our findings from neurochemical measurements made in the selectively-bred rats described earlier in this paper.

5.1. *General procedures*

Male rats of approximately six months of age were used in all studies. For the selectively-bred rats, the animals came from the lines described earlier in this paper. For comparison purposes, we maintain three separate lines of non-selected rats (i.e., randomly-bred or normal rats). One of these non-selected lines has been maintained for as long as our selective breeding program has been underway (referred to hereafter as ''NS-a''), while two other lines of non-selected rats are maintained based on breeding stocks purchased more recently (''NS-b'' and ''NS-c''). The different non-selected lines are maintained, and compared, to determine if ''genetic drift'' has occurred in our original non-selected animals so as to result in abnormal characteristics in this line. For this purpose, each experiment described below included a group of ''NS-a'' rats and also a group of either ''NS-b'' or ''NS-c'' rats (note: the neurochemical data to be presented show no indication of unusual characteristics in the ''NS-a'' line in this regard.) Prior to use in all studies, animals were housed two per cage directly on bedding in standard polypropylene cages where they have access to ad libitum food and water.

Measurement was made of the monoamines norepinephrine [NE], dopamine [DA], and serotonin [5-HT]) as well as their metabolites – 3-methoxy-4 hydroxyphenylglycol [MHPG] for NE, homovanillic acid [HVA] and 3,4-dihydroxyphenylacetic acid [DOPAC] for DA, and 5-hydroxyindoleacidic acid [5-HIAA] for 5-HT – in several brain regions of selectively-bred rats and also in non-selected (i.e., normal) rats for comparison. All animals were sacrificed by decapitation following brief anesthesia with Isoflurane. Following sacrifice, the brain of each animal was rapidly removed from the skull case, the brain cut into 1.0 mm thick sections that were placed onto an ice-cold plate, and various brain regions were then dissected. Tissue samples were placed into tubes and stored at −80 °C for later analysis.

The brain regions dissected for analysis were locus coeruleus (LC), ventral portion of the bed nucleus of the stria terminalis (BNST), hypothalamus (HYP), dorsal hippocampus (HIPP), prefrontal cortex (PFC), striatum (STR), and nucleus accumbens (NAC). Three regions (LC, BNST, NAC) were dissected by using a punch, the remaining regions (HYP, HIPP PFC, STR) were larger and were dissected using an ocular scissors. Approximate wet weights of these tissue samples were (in mg): LC, BNST, and NAC – 3.0–4.0, STR – 15.0–23.0, HYP – 35.0–40.0, HIPP – 45.0–60.0, and PFC – 55.0–70.0. The regions chosen were selected because: LC is the principle noradrenergic cell-body region in the brain and gives rise to the dorsal noradrenergic projection system, ventral BNST is more densely and exclusively innervated by the ventral noradrenergic projection system than any other forebrain region, HYP is responsible for many important vegetative functions and contains all three monoamines being measured, HIPP and PFC are importantly implicated in functions related to psychopathology and monoamines in these brain regions are implicated in these processes, and STR and NAC are the major forebrain projection fields that receive dopaminergic input in the brain.

Monoamines (NE, DA, and 5-HT) and monoamine metabolites (DOPAC, HVA and 5-HIAA) in tissue samples were extracted by ultrasonic disruption of the tissue samples in 0.1 M perchloric acid containing EDTA and metabisulfate as antioxidants. Dihydroxybenzylamine was included as an internal standard. Extracts were centrifuged at high speed and the supernatant analyzed without further processing by automated, reverse phase, ion-pair high pressure liquid chromatography (HPLC) using ESA Coulochem II dual potentiostats and 3 × 100 mm Spherisorb ODS2 columns (Murrough et al. [59]). The use of high sensitivity electrochemical detection with coulometric screening obviated the need for alumina extraction while permitting measurement of a broad spectrum of monoamines and metabolites in a single chromatographic run. Optimal sensitivity at maximal column efficiency was 0.1 picogram (pg), although this sensitivity decays to about 0.5 pg with continued use of the column. Injections onto the HPLC apparatus were done by computer-controlled autosamplers equipped with cooled sample trays; this permitted 24-h operation and better precision (CV < 3%) than is normally achieved by hand. Determination of monoamine and metabolite amounts was done by measurement of the area of the peak obtained in the chromatograph when compared with the internal standard. The size of tissue samples was determined by using the Lowry technique to measure the protein content of the perchlorate pellet remaining after centrifugation, and monoamine and metabolite content was then expressed as pg/mg tissue protein of the sample. When MHPG was measured, the total amount of this metabolite in the sample was determined. This was done by taking an aliquot of the sample and conducting enzymic desulfation to cleave the SO_4 group from the MHPG; this was

followed by an ethyl acetate extraction after which the aliquot was blown dry and resuspended in moble phase for HPLC analysis.

5.2. Description of experiments and results

5.2.1. Neurochemical measurement in SwHi and SwLo rats

Subjects were SwHi ($n = 6$) and SwLo ($n = 7$) rats from generation 40 of the originally-derived SwHi and SwLo lines (referred to here as ''SwHi-a'' and ''SwLo-a''), as well as SwHi ($n = 6$) and SwLo ($n = 6$) rats from generation 27 of parallel lines of SwHi and SwLo rats that had been split off from the original lines at generation 13, moved to another location in the colony, and selectively-bred separately thereafter (referred to here as ''SwHi-b'' and ''SwLo-b''). Together with the SwHi and SwLo rats, two groups of non-selected rats (each group $n = 6$), each group taken from a different line of non-selected rats, including the rats from the original line of non-selected rats (''NS-a'' [generation 40] and ''NS-b''), were also used. For this study, all subjects were simply removed from the colony, sacrificed, brains dissected, and tissue samples frozen. All tissue samples in this study were then processed for neurochemical analysis at the same time.

5.2.2. Neurochemical measurement in SUS and RES rats

Subjects were SUS rats from generations 28 ($n = 22$), 29 ($n = 14$), and 31 ($n = 10$) as well as RES rats from generation 31 ($n = 9$). Insofar as SUS rats came from different generations, sacrifice and tissue collection of these animals necessarily took place periodically over an extended period (i.e., two years). Together with SUS and RES rats, two groups of non-selected rats ($n = 5$ and $n = 6$), each group taken from a different line of non-selected rats, including rats from the original line of non-selected rats (''NS-a'' [generation 36] and NS-c''), were also used. (Tissue collection from the non-selected rats occurred at the same time as tissue collection of SUS generation 29.) For this study, all subjects were simply removed from the colony, sacrificed, brains dissected, and tissue samples frozen. All tissue samples in this study were then processed for neurochemical analysis at the same time.

Of the 14 SUS rats used from generation 29, these rats had been pre-tested for their proclivity to consume alcohol. Eight of these rats were classified as ''alcohol drinkers''; all rats so classified were found to consume more than 4.5 ml/kg body weight of alcohol in a 24-h period when offered the opportunity to drink a 10% alcohol solution. The mean (and S.E.) consumption of alcohol was 6.6 ± 0.5 mg/kg for these animals. Six SUS rats that showed little proclivity to consume alcohol – ''alcohol non-drinkers'' – made up the remaining SUS rats assessed in this generation; all animals so classified were found to consume less than 1.7 mg/kg body weight of alcohol in 24 h when offered the 10% alcohol solution. The mean (and S.E.) consumption of alcohol was 1.3 ± 0.2 mg/kg for these animals. The intent of this alcohol consumption pre-testing was to enable neurochemical measurements to be compared in SUS rats that were ''alcohol drinkers'' vs. ''alcohol non-drinkers.''

5.2.3. Neurochemical measurement in HYPER rats

Two procedures were used. In the first procedure (A), subjects were HYPER rats of generation 28 ($n = 8$) and non-selected rats (NS-c) ($n = 7$). For this study, all subjects were simply removed from the colony, sacrificed, brains dissected, and tissue samples frozen as described above. In the second procedure (B), subjects were HYPER rats of generation 32 ($n = 8$) and non-selected rats from the original line of these animals (NS-a; generation 38) ($n = 7$). In the second procedure, animals were taken from the colony and placed into a novel cage for 75 min prior to sacrifice, dissection, and freezing of tissue samples. This was done to determine how monoaminergic metabolites in brain might be affected by exposure of the animals to a mildly stimulating/stressful situation (i.e., novel cage). All tissue samples in this study (i.e., samples from Procedures A and B) were then processed for neurochemical analysis at the same time.

6. Results

6.1. Results for SwHi and SwLo rats

The results from the neurochemical measurements on two lines of SwHi and SwLo rats together with the two lines of non-selected rats are shown in Table 1. This table shows, for the seven brain regions assessed, the mean and S.E. values for all of the three monoamines measured (NE, DA, 5-HT) as well as for their metabolites (MHPG, HVA, DOPAC, 5-HIAA) and also the ratio of these metabolites to their relevant monoamine as an estimate of turnover (release) of the monoamine.

Two observations can be noted. First, while it might have been anticipated that SwHi and SwLo rats would have differed considerably with respect to DA in the basal ganglia, particularly in the STR but also in the NAC, as DA in these regions has been importantly implicated in mediation of motor activity, the results reveal little difference in the concentration of DA and the principle extracellular metabolite of DA, HVA, in these brain regions. Considering the very large differences in the amount of motor activity in the swim test shown by SwHi and SwLo rats, the findings therefore indicate that the swim-test activity differences shown by these lines are not mediated by differences in the concentration of DA in STR or NAC or in release of this amine, as indicated by HVA, at least under ''resting'' conditions (i.e., animals were measured after removal from the home cage and not in response to the swim test or any challenging stimulus). However, a notable exception to this picture was seen in the prefrontal cortex (PFC). In this brain region, both DA and HVA were significantly higher in SwHi rats than in SwLo rats. Regardless of the fact that the HVA/DA ratio in the PFC did not differ, the findings nevertheless indicate that the amount of DA and DA release (as indicated by HVA levels) in the PFC is higher in SwHi rats than in SwLo rats.

Second, the largest difference in monoamines noted between SwHi and SwLo rats was in the concentration of NE in the hippocampus, where SwHi rats had a higher concentration of NE than did SwLo rats. Also, the mean level of NE in hippocampus of the non-selected rat lines fell between that of the SwHi and SwLo rats, so that the concentration of NE in the hippocampus reflected the ordering of activity shown by these lines of animals in the swim test (i.e., SwHi –most activity, non-selected – mid-range activity, SwLo – least activity). The NE level in the hippocampus of these groups is represented by the bars on the left side of Fig. 9.

Table 1 – Mean (bold) and standard error (non-bold) for concentration of monoamines (norepinephrine [NE], dopamine [DA], serotonin [5-HT]), their metabolites (3-methox-4-hydroxyphenylglycol [MHPG], homovanillic acid [HVA], 3,4-dihyroxyphenylacidic acid [DOPAC], 5-hydoxyindoleacidic acid [5-HIAA]), and metabolite/monoamine ratios in various brain regions from Swim High-active (SwHi), Swim Low-active (SwLo), and non-selected (i.e., non-selectively bred, or normal) rats (NS)

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
Locus coeruleus	NS-a	**26.521**	**5.758**	**0.216**	**3.966**	**0.753**	**2.646**	**0.196**	**0.712**	**16.499**	**10.701**	**0.643**	**0.266**
		±1.628	0.521	0.012	0.390	0.051	0.248	0.016	0.102	0.961	1.455	0.066	0.009
	NS-b	**22.448**	**4.622**	**0.206**	**3.253**	**0.692**	**2.489**	**0.212**	**0.771**	**15.601**	**11.264**	**0.736**	**0.334**
		1.996	0.433	0.006	0.165	0.058	0.271	0.014	0.082	1.345	0.884	0.050	0.020
	SwHi-a	**18.858**[A,C]	**4.580**	**0.249**	**3.153**	**0.660**	**2.068**	**0.223**	**0.665**	**16.043**	**12.222**	**0.754**	**0.326**
		1.238	0.277	0.025	0.356	0.042	0.242	0.027	0.062	1.172	1.281	0.029	0.012
	SwHi-b	**25.806**	**5.169**	**0.204**	**3.629**	**0.847**	**2.516**	**0.233**	**0.690**	**16.783**	**12.267**	**0.735**	**0.300**
		1.961	0.345	0.015	0.265	0.091	0.317	0.017	0.064	1.344	0.935	0.034	0.015
	SwLo-a	**24.213**	**4.906**	**0.207**	**2.581**[A]	**0.644**	**1.623**	**0.254**	**0.623**	**16.662**	**12.482**	**0.747**	**0.295**
		1.845	0.341	0.017	0.225	0.071	0.197	0.026	0.036	0.662	0.730	0.023	0.014
	SwLo-b	**27.969**	**5.148**	**0.187**	**3.319**	**0.691**	**2.349**	**0.211**	**0.706**	**16.336**	**12.310**	**0.768**	**0.300**
		1.845	0.330	0.015	0.277	0.060	0.329	0.017	0.069	0.850	0.876	0.075	0.021
Ventral bed nuc. stria terminalis	NS-a	**43.313**	**3.488**	**0.078**	**24.414**	**2.497**	**5.150**	**0.125**	**0.247**	**11.270**	**7.743**	**0.700**	**0.286**
		4.011	0.710	0.010	5.515	0.328	0.799	0.025	0.041	0.965	0.391	0.033	0.018
	NS-b	**46.217**	**2.823**	**0.063**	**25.669**	**2.250**	**4.858**	**0.101**	**0.204**	**12.521**	**7.446**	**0.616**	**0.308**
		5.009	0.212	0.005	4.049	0.077	0.453	0.018	0.022	0.973	0.509	0.071	0.015
	SwHi-a	**45.804**	**2.634**	**0.057**	**22.303**	**2.263**	**4.390**	**0.123**	**0.218**	**17.073**[B,C]	**8.278**	**0.486**[A]	**0.322**
		3.228	0.285	0.004	4.651	0.077	0.515	0.022	0.021	1.538	0.818	0.029	0.023
	SwHi-b	**37.075**	**3.001**	**0.097**	**35.803**	**3.366**[B,D]	**6.465**	**0.103**	**0.195**	**14.293**	**9.034**[D]	**0.633**	**0.273**
		5.602	0.243	0.022	7.838	0.471	1.014	0.010	0.018	0.964	0.732	0.034	0.007
	SwLo-a	**38.801**	**2.895**	**0.080**	**26.946**	**1.643**	**4.865**	**0.084**	**0.207**	**13.963**	**6.510**	**0.470**[A]	**0.293**
		4.724	0.303	0.012	8.117	0.322	1.237	0.018	0.022	0.995	0.393	0.024	0.007
	SwLo-b	**48.585**	**2.771**	**0.058**	**30.676**	**2.183**	**5.409**	**0.082**	**0.194**	**12.804**	**6.616**	**0.532**	**0.275**
		5.253	0.256	0.004	4.975	0.218	0.510	0.019	0.024	0.947	0.471	0.057	0.011
Hypothalamus	NS-a	**20.125**	**1.439**	**0.072**	**4.720**	**0.639**	**1.009**	**0.137**	**0.208**	**13.701**	**14.735**	**1.079**	**3.576**
		1.623	0.094	0.003	0.541	0.078	0.242	0.010	0.039	0.979	1.337	0.079	0.185
	NS-b	**19.874**	**1.651**	**0.083**	**4.075**	**0.662**	**0.788**	**0.174**	**0.197**	**13.880**	**16.285**	**1.186**	**3.350**
		0.763	0.071	0.004	0.581	0.035	0.133	0.022	0.021	0.882	1.071	0.087	0.104
	SwHi-a	**22.550**	**1.393**	**0.062**[A]	**4.149**	**0.693**	**1.189**	**0.171**	**0.277**	**14.871**	**15.436**	**1.039**	**3.265**
		1.077	0.176	0.008	0.461	0.071	0.274	0.018	0.041	0.523	0.840	0.045	0.157
	SwHi-b	**22.510**	**1.516**	**0.068**[A]	**5.727**	**0.988**[D]	**1.857**	**0.183**	**0.306**	**14.187**	**15.824**	**1.126**	**3.153**
		1.315	0.095	0.003	1.157	0.144	0.528	0.015	0.041	0.938	0.943	0.066	0.258
	SwLo-a	**20.658**	**1.334**	**0.064**[A]	**4.176**	**0.599**	**1.031**	**0.147**	**0.228**	**12.974**	**13.140**	**1.021**	**3.152**
		1.007	0.104	0.003	0.542	0.059	0.260	0.007	0.029	1.122	1.197	0.064	0.292
	SwLo-b	**23.153**	**1.506**	**0.066**[A]	**4.226**	**0.608**	**1.099**	**0.147**	**0.259**	**14.897**	**13.515**	**0.956**	**3.037**
		2.237	0.111	0.002	0.824	0.116	0.328	0.016	0.054	1.822	0.846	0.099	0.113
Hippocampus	NS-a	**4.087**	**0.920**	**0.225**	**0.171**	**0.231**	**0.158**	**1.778**	**1.406**	**4.864**	**4.872**	**1.003**	**4.638**
		0.160	0.055	0.011	0.036	0.021	0.051	0.445	0.762	0.312	0.415	0.065	0.175

Table 1 (*Continued*)

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
	NS-b	**3.721**	**0.934**	**0.255**	**0.124**	**0.218**	**0.087**	**1.859**	**0.750**	**5.061**	**5.799**	**1.152**	**4.451**
		0.214	0.039	0.016	0.013	0.021	0.013	0.221	0.136	0.249	0.394	0.074	0.233
	SwHi-a	**4.437**^{A,D}	**0.814**	**0.183**^{A}	**0.143**	**0.256**	**0.137**	**1.859**	**1.066**	**5.221**	**4.851**	**0.923**	**4.698**
		0.305	0.061	0.003	0.014	0.021	0.051	0.218	0.451	0.341	0.530	0.077	0.231
	SwHi-b	**5.417**^{B,D}	**1.232**^{B,D}	**0.229**	**0.211**	**0.332**^{B,D}	**0.065**	**2.025**	**0.443**	**5.865**	**5.620**	**0.993**	**4.328**
		0.268	0.173	0.032	0.049	0.032	0.019	0.537	0.233	0.629	0.259	0.072	0.148
	SwLo-a	**3.587**	**0.777**	**0.217**	**0.107**	**0.209**	**0.123**	**2.202**	**1.346**	**4.441**	**4.161**	**0.942**	**4.649**
		0.101	0.031	0.009	0.015	0.009	0.024	0.312	0.433	0.222	0.423	0.094	0.193
	SwLo-b	**3.476**	**0.854**	**0.247**	**0.159**	**0.202**	**0.102**	**1.409**	**0.740**	**4.953**	**4.750**	**0.973**	**4.200**
		0.125	0.004	0.008	0.025	0.012	0.019	0.219	0.144	0.298	0.317	0.088	0.332
Prefrontal cortex	NS-a	**4.093**	**1.335**	**0.327**	**2.492**	**0.903**	**0.927**	**0.373**	**0.367**	**10.102**	**5.068**	**0.504**	**6.555**
		0.121	0.050	0.015	0.254	0.040	0.148	0.022	0.036	0.288	0.247	0.029	0.209
	NS-b	**4.453**	**1.654**	**0.372**	**2.711**	**1.004**	**1.053**	**0.380**	**0.381**	**10.578**	**5.876**	**0.560**	**6.657**
		0.102	0.046	0.012	0.291	0.066	0.218	0.022	0.061	0.539	0.450	0.045	0.339
	SwHi-a	**4.203**	**1.341**^{A}	**0.320**^{A}	**3.473**	**0.871**^{D}	**1.257**^{B}	**0.260**	**0.361**	**11.836**	**6.045**	**0.512**	**6.224**
		0.134	0.028	0.008	0.332	0.034	0.128	0.020	0.009	0.255	0.104	0.010	0.276
	SwHi-b	**4.796**^{A}	**1.485**	**0.310**^{A}	**3.718**^{A,C}	**1.052**^{D}	**1.145**^{B}	**0.291**	**0.334**	**10.082**	**5.799**	**0.577**^{C}	**6.589**
		0.179	0.053	0.007	0.448	0.082	0.151	0.020	0.051	0.181	0.153	0.022	0.231
	SwLo-a	**4.329**^{C}	**1.467**	**0.339**	**2.390**	**0.690**^{B}	**0.777**^{B}	**0.294**	**0.327**	**11.692**	**6.200**^{A}	**0.532**	**6.235**
		0.065	0.070	0.014	0.199	0.038	0.059	0.015	0.014	0.380	0.269	0.024	0.276
	SwLo-b	**5.087**^{B,C}	**1.481**	**0.293**^{A}	**2.627**	**0.675**^{B}	**0.682**^{B}	**0.258**	**0.254**	**11.827**	**5.071**	**0.436**^{A}	**7.154**
		0.151	0.057	0.015	0.144	0.030	0.131	0.009	0.036	0.687	0.181	0.029	0.273
Striatum	NS-a	**0.993**	**0.839**	**0.845**	**160.816**	**10.644**	**21.477**	**0.067**	**0.133**	**9.684**	**8.896**	**0.941**	**1.631**
		0.027	0.050	0.046	12.289	0.624	2.018	0.003	0.005	0.651	0.309	0.072	0.133
	NS-b	**0.969**	**0.874**	**1.262**	**162.541**	**13.227**	**21.473**	**0.081**	**0.131**	**10.597**	**10.339**	**0.989**	**1.668**
		0.228	0.071	0.402	11.696	1.402	2.031	0.005	0.005	1.045	0.858	0.044	0.144
	SwHi-a	**1.066**	**0.774**^{C}	**0.755**	**153.584**	**10.340**	**19.040**	**0.068**	**0.124**	**11.926**	**9.122**	**0.767**^{A}	**1.725**
		0.106	0.033	0.063	5.328	0.335	1.209	0.003	0.005	0.696	0.518	0.025	0.045
	SwHi-b	**1.090**	**0.878**	**0.819**	**154.807**	**12.088**	**21.982**	**0.079**^{C}	**0.143**	**11.670**	**10.200**^{C}	**0.888**	**1.501**
		0.071	0.029	0.048	5.652	0.534	1.794	0.004	0.013	0.747	0.402	0.058	0.041
	SwLo-a	**1.119**	**0.959**	**0.942**	**166.959**	**10.556**	**18.119**	**0.063**^{A}	**0.108**	**10.146**	**7.701**^{A}	**0.775**^{A}	**1.483**
		0.168	0.066	0.100	9.368	0.739	1.379	0.003	0.005	1.077	0.556	0.033	0.048
	SwLo-b	**0.901**	**0.998**	**1.117**	**151.184**	**10.975**	**19.963**	**0.073**	**0.133**	**10.792**	**8.628**	**0.805**^{A}	**1.644**
		0.044	0.037	0.054	6.895	0.622	1.686	0.004	0.011	0.598	0.322	0.026	0.097
Nucleus accumbens	NS-a	**2.157**	**1.562**	**0.930**	**74.118**	**7.172**	**20.736**	**0.097**	**0.277**	**11.173**	**9.065**	**0.829**	**0.216**
		0.569	0.189	0.211	6.070	0.698	2.183	0.004	0.009	0.787	0.641	0.071	0.006
	NS-b	**1.913**	**1.777**	**1.218**	**74.937**	**8.183**	**20.387**	**0.110**	**0.274**	**13.026**	**9.047**	**0.704**	**0.242**
		0.487	0.161	0.248	5.127	0.445	1.141	0.005	0.010	0.993	1.028	0.081	0.012
	SwHi-a	**1.390**	**1.509**	**1.332**	**74.521**	**7.287**	**18.477**	**0.097**	**0.246**	**15.995**	**10.906**	**0.737**	**0.235**
		0.265	0.187	0.287	3.908	0.564	2.251	0.004	0.025	1.946	0.492	0.095	0.007

Table 1 (Continued)

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
	SwHi-b	**2.143**	**1.809**	**1.007**	**80.856**	**9.880**^{B,D}	**22.552**	**0.123**	**0.278**	**14.741**	**11.339**	**0.807**	**0.207**
		0.341	0.309	0.279	3.458	0.438	1.590	0.005	0.016	2.149	0.955	0.071	0.004
	SwLo-a	**1.396**	**2.110**	**1.755**	**64.794**	**5.830**^{A}	**16.711**	**0.098**	**0.262**	**11.780**	**9.128**	**0.808**	**0.212**
		0.215	0.504	0.557	5.625	0.405	1.447	0.017	0.019	1.483	0.659	0.048	0.015
	SwLo-b	**1.824**	**1.434**	**0.952**	**73.034**	**7.804**	**20.644**	**0.108**	**0.285**	**11.826**	**10.022**	**0.911**	**0.205**
		0.555	0.144	0.136	3.744	0.280	1.107	0.006	0.018	1.278	0.921	0.163	0.007

Concentrations shown are ng/mg protein. Group designations are: NS-a – non-selected, generation 40 of original line bred along with original line of SwHi and SwLo [$n = 6$]; NS-b – non-selected, a different line of non-selected rats originated several generations after the original line of NS rats [$n = 6$]; SwHi-a – Swim High-active, generation 40 of the original line [$n = 7$]; SwHi-b – Swim High-active, generation 27 of a line separated from the original SwHi line at generation 13 and selectively bred separately thereafter [$n = 6$]; SwLo-a – Swim Low-active, generation 40 of the original line [$n = 6$]; SwLo-b – generation 27 of a line separated from the original SwLo line at generation 13 and selectively bred separately thereafter [$n = 6$]. **Statistical significance:** statistically significant differences between individual group means are designated by superscript letters. In each brain region and for each monoamine or metabolite in that brain region, a one-way analysis of variance was conducted for the six groups, followed by comparisons between individual groups done by the Newman–Keuls method. A group mean that was found to be significantly different (at least $p < .05$) from other groups is designated as follows: A = differs from one of the non-selected (NS) group; B = differs from both of the non-selected (NS) groups; C = differs from one of the groups of the opposite selectively-bred line (i.e., if noted for a SwHi group, this group differs from one of the SwLo groups; if noted for a SwLo group, this group differs from one of the SwHi groups); and D = differs from both of the groups of the opposite selectively-bred line.

Fig. 9 – Concentration of norepinephrine (ng/mg protein) in the hippocampus of all selectively-bred lines of rats and in all non-selectively bred lines included for comparison. Groups were: non-selected (NS), from the original line of rats bred non-selectively in parallel with the first selectively-bred lines that were generated (NS-a) as well as two non-selected lines begun later (NS-b and NS-c); Swim High-active (SwHi) and Swim Low-active (SwLo), from the original line of SwHi and SwLo (SwHi-a and SwLo-a) as well as from parallel lines separated at generation 13 and bred separately thereafter (SwHi-b and SwLo-b); Hyperactive (HYPER) from generations 28 (HYPER-G28) and 32 (HYPER-G32); Swim-test Susceptible (SUS) from generations 28 (SUS-G28), 29 (SUS-G29) and 31 (SUS-G31); and Swim-test Resistant (RES) from generation 31 (RES-G31). Means and standard errors are shown; values are ng/mg protein of tissue sample. (*) Differs significantly (at least $p < .05$) from contrasting rat line (i.e., all SwLo groups differ from all SwHi, and all SUS differ from RES). Also, SwLo groups differ significantly from NS-a but not from NS-b, while all SUS groups differ significantly from NS-a and NS-c.

6.2. *Results for SUS and RES rats*

The results from the neurochemical measurements on three generations of SUS rats (generations 28, 29, and 31), one corresponding generation of RES rats (generation 31), and two lines of non-selected rats are shown in Table 2. This table shows, as was presented for SwHi and SwLo rats, the mean and S.E. values for all of the three monoamines measured as well as for their metabolites and also the relevant metabolite/monoamine ratio.

The most notable differences between SUS, RES, and non-selected rats were seen with respect to DA in forebrain regions (STR, NAC, PFC). For the concentration of DA in STR, all three generations of SUS rats had levels that were significantly lower than were seen in the two lines of non-selected rats. In contrast, the RES rats had a significantly higher concentration of DA in the STR than did the two lines of non-selected rats. Thus, ability of a stressor to produce a decrease in swim-test

Table 2 – Mean (bold) and standard error (non-bold) for concentration of monoamines (norepinephrine [NE], dopamine [DA], serotonin [5-HT]), their metabolites (3-methox-4-hydroxyphenylglycol [MHPG], homovanillic acid [HVA], 3,4-dihyroxyphenylacidic acid [DOPAC], 5-hydoxyindoleacidic acid [5-HIAA]), and metabolite/monoamine ratios in various brain regions from Swim-test Susceptible (SUS), Swim-test Resistant (RES), and non-selected (i.e., non-selectively bred, or normal) rats (NS)

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
Locus coeruleus	NS-a	**24.254**	**5.434**	**0.228**	**2.705**	**0.546**	**2.763**	**0.207**	**1.021**	**14.446**	**12.668**	**0.879**	**0.300**
		1.420	0.255	0.019	0.124	0.101	0.367	0.046	0.121	0.754	0.527	0.015	0.015
	NS-c	**18.193**	**4.999**	**0.275**	**2.584**	**0.815**	**2.551**	**0.308**	**1.027**	**11.960**	**11.826**	**1.008**	**0.288**
		0.390	0.333	0.017	0.238	0.215	0.083	0.063	0.089	0.707	1.776	0.172	0.008
	SUS-G28	**15.049**^{B}	**4.316**	**0.291**^{A}	**2.406**	**0.612**	**2.526**	**0.271**	**1.106**	**10.220**^{A}	**13.228**	**1.426**^{A}	**0.292**
		0.595	0.172	0.011	0.122	0.042	0.145	0.024	0.093	0.696	0.770	0.131	0.006
	SUS-G29	**16.941**^{A}	**5.131**	**0.307**^{A}	**2.388**	**0.490**	**2.587**	**0.254**	**1.284**	**11.529**	**13.665**	**1.208**	**0.206**
		0.743	0.205	0.014	0.224	0.053	0.112	0.056	0.201	0.576	0.596	0.060	0.008
	SUS-G31	**16.667**^{A}	**5.105**	**0.309**^{A}	**2.655**	**0.613**	**2.436**	**0.257**	**1.033**	**13.157**	**9.402**	**0.716**	**0.237**
		0.614	0.241	0.016	0.254	0.100	0.118	0.055	0.165	0.462	0.670	0.043	0.009
	RES-G31	**20.095**^{A,E}	**4.414**	**0.219**^{E}	**3.353**^{D}	**0.364**^{A}	**2.246**	**0.109**	**0.680**	**16.237**^{A,E}	**11.142**	**0.688**^{C}	**0.247**
		0.574	0.432	0.020	0.220	0.047	0.167	0.012	0.048	0.433	0.737	0.046	0.013
Ventral bed nuc. stria terminalis	NS-a	**41.614**	**3.243**	**0.082**	**25.453**	**3.740**	**6.041**	**0.152**	**0.255**	**11.025**	**10.901**	**0.993**	**0.322**
		3.773	0.274	0.012	4.060	0.416	0.686	0.008	0.044	0.331	0.663	0.071	0.017
	NS-c	**41.577**	**3.374**	**0.084**	**26.805**	**4.232**	**4.616**	**0.168**	**0.190**	**10.798**	**10.501**	**1.021**	**0.301**
		1.773	0.385	0.014	6.577	0.766	0.953	0.010	0.026	1.667	0.954	0.077	0.020
	SUS-G28	**41.898**	**2.761**	**0.069**	**26.708**	**4.090**	**5.626**	**0.164**	**0.233**	**10.793**	**10.437**	**0.999**	**0.327**
		2.393	0.170	0.005	2.401	0.307	0.333	0.011	0.016	0.393	0.366	0.051	0.011
	SUS-G29	**37.530**	**3.812**	**0.107**	**25.504**	**4.192**	**4.771**	**0.181**	**0.195**	**10.796**	**10.383**	**0.999**	**0.225**
		1.773	0.151	0.010	2.907	0.334	0.525	0.015	0.014	0.620	0.402	0.059	0.003
	SUS-G31	**43.436**	**4.215**^{A}	**0.098**	**15.523**	**3.350**	**2.763**^{A}	**0.285**^{B}	**0.199**	**9.863**	**11.072**	**1.141**	**0.242**
		1.341	0.136	0.004	3.275	0.343	0.422	0.048	0.014	0.499	0.596	0.070	0.005
	RES-G31	**42.489**	**3.591**	**0.088**	**13.595**	**2.529**	**4.610**	**0.207**	**0.465**	**9.433**	**12.245**	**1.318**^{B,D}	**0.273**
		3.139	0.206	0.008	2.421	0.240	1.121	0.023	0.195	0.750	0.836	0.055	0.012
Hypothalamus	NS-a	**21.672**	**1.307**	**0.060**	**4.624**	**0.371**	**0.739**	**0.081**	**0.160**	**13.710**	**11.220**	**0.826**	**4.025**
		0.756	0.050	0.001	0.260	0.034	0.056	0.007	0.010	0.776	0.235	0.033	0.160
	NS-c	**20.733**	**1.327**	**0.064**	**4.062**	**0.410**	**0.729**	**0.101**	**0.182**	**13.300**	**10.878**	**0.830**	**4.125**
		0.696	0.046	0.002	0.317	0.039	0.057	0.007	0.013	0.584	0.617	0.071	0.220
	SUS-G28	**21.890**	**1.246**	**0.057**	**4.224**	**0.415**	**0.669**	**0.099**	**0.164**	**12.962**	**9.493**	**0.740**	**4.369**
		0.657	0.032	0.001	0.358	0.043	0.040	0.005	0.006	0.444	0.310	0.021	0.121
	SUS-G29	**20.340**	**1.138**^{B}	**0.056**	**4.026**	**0.337**	**0.571**	**0.086**	**0.141**^{A}	**13.058**	**9.690**	**0.744**	**4.205**
		0.488	0.018	0.002	0.223	0.014	0.052	0.005	0.008	0.183	0.328	0.026	0.095
	SUS-G31	**20.452**	**1.198**	**0.059**	**4.300**	**0.352**	**0.563**	**0.081**	**0.129**^{A}	**11.815**	**13.117**^{B}	**1.115**^{B}	**3.701**
		0.700	0.020	0.002	0.301	0.042	0.092	0.005	0.014	0.310	0.469	0.044	0.105
	RES-G31	**23.766**^{D}	**1.151**^{B}	**0.049**^{B,E}	**4.673**	**0.237**^{C}	**0.517**	**0.051**^{B,E}	**0.112**^{B,C}	**16.309**^{B,E}	**14.619**^{B,E}	**0.904**^{C}	**3.586**
		0.883	0.031	0.002	0.175	0.016	0.023	0.004	0.007	0.409	0.702	0.056	0.098
Hippocampus	NS-a	**4.354**	**1.042**	**0.240**	**0.229**	**0.110**	**0.453**	**0.495**	**2.384**	**4.522**	**5.731**	**1.264**	**4.622**
		0.201	0.059	0.012	0.053	0.031	0.030	0.109	0.489	0.082	0.562	0.115	0.184

Table 2 (*Continued*)

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
	NS-c	**4.241**	**0.980**	**0.234**	**0.170**	**0.043**	**0.367**	**0.271**	**2.317**	**4.951**	**5.890**	**1.244**	**4.467**
		0.291	0.063	0.017	0.023	0.005	0.012	0.037	0.242	0.421	0.187	0.138	0.144
	SUS-G28	**3.144**^{B}	**0.877**^{A}	**0.280**^{B}	**0.144**	**0.068**	**0.466**	**0.566**	**3.720**	**4.223**	**7.529**	**1.942**^{B}	**5.026**
		0.061	0.022	0.008	0.013	0.008	0.046	0.077	0.468	0.238	0.425	0.181	0.153
	SUS-G29	**3.501**^{B}	**0.823**^{B}	**0.237**	**0.226**	**0.116**	**0.305**	**0.588**	**1.853**	**6.638**^{B}	**5.062**	**0.787**	**4.892**
		0.094	0.015	0.006	0.040	0.040	0.021	0.139	0.271	0.252	0.366	0.070	0.144
	SUS-G31	**3.284**^{B}	**0.931**	**0.286**^{B}	**0.149**	**0.015**	**0.428**	**0.104**	**2.999**	**5.396**	**6.269**	**1.172**	**5.149**
		0.145	0.026	0.009	0.013	0.010	0.036	0.069	0.266	0.150	0.385	0.083	0.145
	RES-G31	**4.289**^{E}	**0.965**^{C}	**0.225**^{D}	**0.189**	**0.031**	**0.321**	**0.153**^{D}	**1.858**^{C}	**6.349**^{B,D}	**7.012**	**1.112**^{C}	**4.368**
		0.139	0.043	0.006	0.021	0.017	0.033	0.082	0.273	0.188	0.304	0.058	0.111
Prefrontal cortex	NS-a	**4.207**	**1.435**	**0.349**	**2.720**	**0.755**	**1.365**	**0.299**	**0.513**	**9.183**	**6.517**	**0.774**	**6.588**
		0.272	0.028	0.030	0.412	0.037	0.163	0.036	0.032	1.431	0.433	0.106	0.541
	NS-c	**4.598**	**1.548**	**0.337**	**2.722**	**0.924**	**1.394**	**0.361**	**0.527**	**8.803**	**5.195**	**0.608**	**7.099**
		0.099	0.110	0.024	0.330	0.059	0.138	0.040	0.046	0.639	0.142	0.052	0.259
	SUS-G28	**3.365**^{B}	**1.509**	**0.459**	**2.045**	**0.772**	**1.120**	**0.393**	**0.543**	**8.686**	**5.527**^{A}	**0.650**^{A}	**6.358**
		0.149	0.103	0.035	0.141	0.038	0.120	0.016	0.028	0.265	0.165	0.030	0.141
	SUS-G29	**3.382**^{B}	**1.313**	**0.389**	**2.082**	**0.722**	**0.901**	**0.355**	**0.438**	**11.120**^{B}	**5.155**^{A}	**0.466**^{A}	**6.250**
		0.046	0.028	0.009	0.145	0.036	0.056	0.016	0.014	0.278	0.120	0.013	0.188
	SUS-G31	**3.900**^{B}	**1.199**	**0.311**	**2.460**	**0.869**	**0.934**	**0.367**	**0.392**^{B}	**10.130**	**5.818**^{A}	**0.578**^{A}	**6.257**
		0.143	0.036	0.015	0.181	0.049	0.045	0.028	0.023	0.306	0.096	0.016	0.170
	RES-G31	**4.884**^{A,E}	**1.308**	**0.268**^{C}	**3.631**^{E}	**0.586**^{A,C}	**1.075**	**0.180**^{B,E}	**0.309**^{B,D}	**10.030**	**5.494**^{A}	**0.557**^{A}	**6.284**
		0.171	0.044	0.002	0.665	0.048	0.147	0.019	0.013	0.575	0.235	0.028	0.137
Striatum	NS-a	**0.759**	**0.566**	**0.754**	**159.140**	**10.995**	**21.017**	**0.069**	**0.132**	**8.830**	**9.347**	**1.064**	**2.365**
		0.064	0.044	0.047	5.943	0.615	1.273	0.004	0.004	0.740	0.755	0.046	0.165
	NS-c	**0.562**	**0.682**	**1.215**	**147.402**	**11.351**	**18.644**	**0.077**	**0.126**	**7.471**	**8.056**	**1.094**	**2.309**
		0.034	0.119	0.190	4.036	0.901	0.664	0.005	0.002	0.479	1.001	0.131	0.086
	SUS-G28	**0.701**	**0.595**	**0.885**^{A}	**100.641**^{B}	**8.232**^{B}	**14.182**^{B}	**0.082**^{A}	**0.141**	**9.841**^{A}	**8.830**	**0.934**	**2.408**
		0.045	0.030	0.047	3.195	0.320	0.857	0.002	0.006	0.349	0.398	0.064	0.084
	SUS-G29	**0.861**^{A}	**0.503**	**0.624**^{A}	**126.766**^{B}	**10.222**	**14.694**^{B}	**0.080**^{A}	**0.115**	**11.837**^{B}	**8.209**	**0.712**^{B}	**2.322**
		0.060	0.009	0.047	3.971	0.483	0.930	0.003	0.006	0.608	0.185	0.031	0.053
	SUS-G31	**0.771**	**0.513**	**0.697**^{A}	**132.310**^{B}	**11.350**	**13.411**^{B}	**0.086**^{A}	**0.102**^{B}	**10.228**^{A}	**7.566**	**0.752**^{B}	**2.318**
		0.069	0.025	0.054	4.834	0.511	0.518	0.003	0.003	0.702	0.380	0.033	0.068
	RES-G31	**0.613**	**0.547**	**0.964**^{A,C}	**182.444**^{B,E}	**9.193**	**14.812**^{B}	**0.050**^{B,E}	**0.081**^{B,E}	**8.082**^{C}	**9.219**	**1.146**^{D}	**1.837**
		0.069	0.024	0.094	5.056	0.467	0.632	0.002	0.003	0.285	0.207	0.022	0.109
Nucleus accumbens	NS-a	**2.802**			**76.524**	**9.647**	**25.142**	**0.128**	**0.331**	**14.283**	**8.748**	**0.649**	**0.288**
		0.315			4.159	0.210	1.110	0.008	0.014	4.409	2.116	0.040	0.006
	NS-c	**2.508**			**72.542**	**8.887**	**21.159**	**0.125**	**0.302**	**15.605**	**8.401**	**0.566**	**0.303**
		0.305			5.795	0.556	1.100	0.009	0.030	3.526	1.592	0.042	0.006
	SUS-G28	**2.264**			**62.464**	**8.141**^{A}	**18.308**^{B}	**0.135**	**0.309**	**9.940**	**7.732**	**0.841**^{A}	**0.289**
		0.177			3.320	0.279	0.571	0.005	0.017	0.979	0.586	0.056	0.005

Table 2 (*Continued*)

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
	SUS-G29	**1.777**			**68.386**	**7.493**^{A}	**13.181**^{B}	**0.111**	**0.196**^{B}	**15.949**	**10.020**	**0.632**	**0.231**
		0.121			3.717	0.338	0.874	0.004	0.013	0.466	0.181	0.014	0.004
	SUS-G31	**2.387**			**56.985**^{A}	**7.035**^{B}	**14.633**^{B}	**0.124**	**0.259**	**11.673**	**9.455**	**0.827**^{A}	**0.250**
		0.308			2.704	0.441	0.572	0.006	0.008	0.576	0.242	0.042	0.006
	RES-G31	**2.662**			**83.403**^{D}	**6.578**^{B}	**17.009**^{B,C}	**0.080**^{B,E}	**0.205**^{B,C}	**14.835**	**11.179**^{C}	**0.777**	**0.262**
		0.423			3.228	0.375	1.064	0.006	0.012	0.989	0.264	0.047	0.012

Concentrations shown are ng/mg protein. Group designations are: NS-a – non-selected, generation 36 of the original line of non-selectively bred rats bred maintained with lines of selectively-bred rats [n = 5]; NS-c – non-selected, a different line of non-selected rats originated several generations after the original line of NS rats [n = 6]; SUS-G28 – Swim-test Susceptible, generation 28 of this line [n = 22]; SUS-G29 – Swim-test Susceptible, generation 29 of this line [n = 14]; SUS-G31 – Swim-test Susceptible, generation 31 of this line [n = 10]; RES-G31 – Swim-test Resistant, generation 31 of this line [n = 9]. **Statistical significance**: statistically significant differences between individual group means are designated by superscript letters. In each brain region and for each monoamine or metabolite in that brain region, a one-way analysis of variance was conducted for the six groups, followed by comparisons between individual groups done by the Newman–Keuls method. A group mean that was found to be significantly different (at least $p < .05$) from other groups is designated as follows: A = differs from one of the non-selected (NS) groups; B = differs from both of the non-selected (NS) groups; C = differs from one of the SUS groups; D = differs from two of the SUS groups; and E = differs from all three of the SUS groups (symbols C, D, and E, which designate differences between the SUS and RES lines, are applied to the RES group).

activity was correlated with the concentration of DA in the STR – RES rats, which are the most resistant, had the highest concentration of DA in the STR; non-selected rats, which fall between RES and SUS rats in resistance, had corresponding mid-range concentrations of DA in STR; and SUS rats, which are the most susceptible, had the lowest concentrations of DA in STR. (Interestingly, the lower levels of DA in STR of SUS rats was accompanied by higher levels of 5-HT in this brain region.) For DA concentration in the NAC and PFC, the groups arranged themselves similarly, although the differences were less clear-cut than in the STR. Regarding HVA, SUS rats tended to have lower levels than non-selected rats; however, the most noteworthy effect here was that RES rats, which clearly had the highest concentrations of DA, also had lower concentrations of HVA than non-selected rats and, in almost all instances, than SUS rats as well, indicating that the release of DA by RES rats in forebrain DA regions was, despite higher DA levels in RES rats, less than occurred in non-selected and SUS rats.

Another notable difference was the low concentration of NE seen in the hippocampus of SUS rats. Whereas RES and non-selected rats did not differ in this respect, SUS rats of all three generations measured had lower levels of NE in the hippocampus than did the RES and non-selected rats (see Fig. 9).

6.3. *Alcohol drinks vs. non-drinkers*

As explained above, SUS rats from generation 29 were also classified as ''alcohol drinkers'' and ''alcohol non-drinkers.'' Eight SUS rats consumed considerable amounts of alcohol while six SUS rats were chosen that did not. Concentrations of NE, DA, and 5-HT, their metabolites, and relevant metabolite/monoamine ratios for the rats characterized as ''alcohol drinkers'' and ''alcohol non-drinkers'' are shown in Fig. 10. The most consistent differences were seen with respect to DA in the NAC and PFC, two major projection fields of mesocorticolimbic DA system (i.e., cell bodies in VTA projecting to NAC and PFC). Alcohol drinkers showed lower concentrations of DA and HVA in these brain regions, which, despite this resulting in slightly higher HVA/DA ratios, nevertheless indicates that DA release is reduced in SUS rats that are alcohol drinkers compared to SUS rats that are not alcohol drinkers. Interestingly, drinkers also differed from non-drinkers with respect to 5-HT in the NAC, with evidence for reduced serotonin release in the NAC of drinkers. Differences in the NE and 5 HT were also seen in the ventral BN. For NE, BN ventral bed nucleus is the forebrain region most exclusively innervated by the ventral noradrenergic projection system, and therefore the results suggest that there is a different level of activity of the ventral bundle NE system in drinkers vs. non-drinkers.

6.4. *Results for HYPER rats*

The results from the neurochemical measurements of HYPER rats under two conditions, one with sacrifice occurring simply after removal from the home cage (HYPER generation 28 and NS-c for comparison) and the other after the animals had been exposed to a novel cage for 75 min cage prior to sacrifice (HYPER generation 32 and NS-a for comparison) are shown in

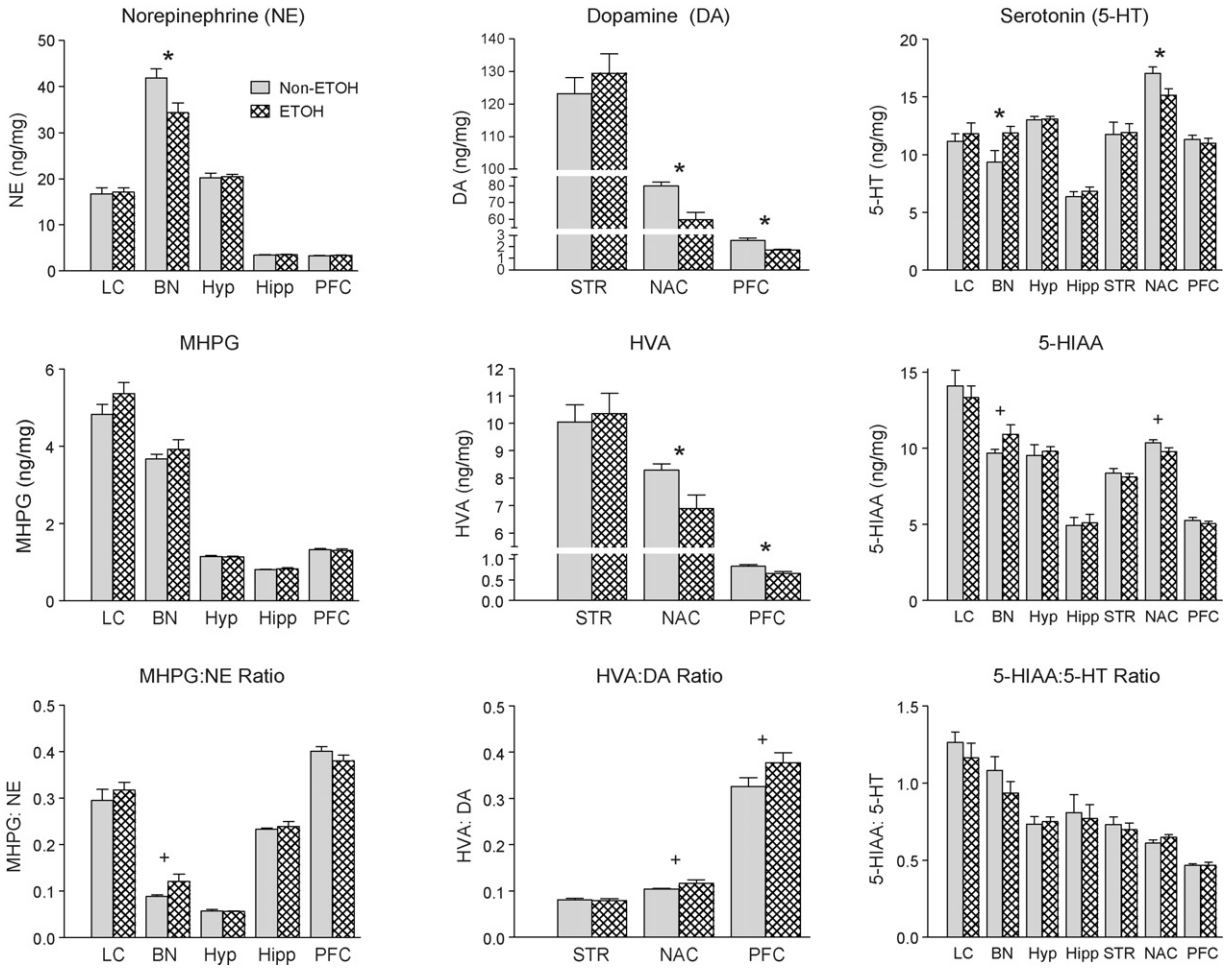

Fig. 10 – In Swim-test Susceptible rats from generation 29 that were "alcohol drinkers" (ETOH) and "alcohol non-drinkers" (Non-ETOH), the concentration of norepinephrine (NE), dopamine (DA) and serotonin (5-HT), their respective principal extracellular metabolites (MHPG, HVA, 5-HIAA), and the ratio of the concentration of metabolite to the concentration of amine in various brain regions. For monoamine and metabolites, values are ng/mg protein of the tissue sample. Brain regions: locus coeruleus (LC), ventral part of the bed nucleus of the stria terminalis (BN), hypothalamus (Hyp), hippocampus (Hipp), prefrontal cortex (PFC), striatum (STR), and nucleus accumbens (NAC). Means and standard errors are shown. (*) Significant difference ($p < .05$) between ETOH and Non-ETOH; (+) difference between ETOH and Non-ETOH approaches significance ($p < .10$).

Table 3. This table shows, as was presented for the other selectively-bred lines addressed in this paper, the mean and S.E. values for all of the three monoamines measured as well as for their metabolites and also the relevant metabolite/monoamine ratio (MHPG is shown only in Part B as it was only measured in animals that had been exposed to the novel environment for 75 min).

Fig. 11 shows, for the animals exposed to the novel environment and for which MHPG was therefore measured, the concentrations of NE and DA seen in brain regions of interest as well concentrations of MHPG and HVA and the relevant ratios of metabolite to monoamine. Important differences shown in this figure were also seen for the HYPER vs. non-selected rats whose results are shown in top part of Table 3 (i.e., animals not exposed to the novel environment prior to sacrifice), and therefore represent consistent differences between HYPER and normal rats. As can be seen from Fig. 11 and Table 3, NE concentrations were generally higher throughout the brain in HYPER rats than in non-selected rats. Fig. 11 shows that, in contrast, MHPG levels were lower, so that MHPG/NE ratios were notably lower in the HYPER rats than in non-selected rats. These findings point to elevated brain NE levels in HYPER rats but widespread reduced release of NE throughout the brain in HYPER rats.

Marked differences in DA concentration and metabolism are shown in Fig. 11, and some similar differences also can be seen in the top part of Table 3. DA concentration in STR was less in HYPER rats than in non-selected rats. The most dramatic effect related to forebrain DA, however, was the reduced HVA levels in STR and NAC of HYPER rats (also seen in the PFC in rats exposed to the novel environment prior to sacrifice [i.e., in bottom part of Table 3; shown in Fig. 11]). The reduced HVA was so marked in these brain regions of HYPER rats that the HVA/DA ratio was in some instances significantly

Table 3 – Mean (bold) and standard error (non-bold) for, at top, concentration of monoamines (norepinephrine [NE], dopamine [DA], serotonin [5-HT]), the metabolites for DA (homovanillic acid [HVA] and 3,4-dihyroxyphenylacidic acid [DOPAC]) and serotonin (5-hydoxyindoleacidic acid [5-HIAA]), and metabolite/monoamine ratios in various brain regions from Hyperactive rats (HYPER) and non-selected (i.e., non-selectively bred, or normal) rats (NS); At bottom, same as at top) except measurement of the NE metabolite 3-methox-4-hydroxyphenylglycol [MHPG] and the MHPG/NE ratio is also included

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
Locus coeruleus	NS-c	**22.883**			**2.853**	**0.483**	**1.702**	**0.182**	**0.625**	**12.540**	**9.865**	**0.784**	**0.456**
		1.425			0.304	0.050	0.160	0.025	0.067	0.915	1.243	0.075	0.021
	HYPER-G28	**33.682**[A]			**3.158**	**0.361**[A]	**1.811**	**0.115**[A]	**0.573**	**17.755**	**10.537**	**0.633**	**0.401**
		2.619			0.164	0.020	0.142	0.007	0.032	2.260	0.813	0.069	0.018
Ventral bed nuc. stria terminalis	NS-c	**44.898**			**20.129**	**2.998**	**6.747**	**0.158**	**0.356**	**11.770**	**10.067**	**0.897**	**0.393**
		1.551			3.444	0.361	1.158	0.016	0.059	0.734	0.901	0.129	0.023
	HYPER-G28	**52.618**[A]			**20.049**	**2.005**[A]	**4.350**	**0.108**[A]	**0.248**	**15.337**	**8.921**	**0.621**	**0.375**
		1.870			3.567	0.191	0.441	0.009	0.043	1.625	0.348	0.067	0.016
Hypothalamus	NS-c	**20.163**			**3.859**	**0.321**	**0.740**	**0.083**	**0.178**	**13.693**	**10.836**	**0.792**	**3.817**
		1.326			0.409	0.056	0.164	0.010	0.027	0.586	0.556	0.023	0.277
	HYPER-G28	22.434			3.746	0.276	0.577	0.072	0.140	15.500	10.724	0.713	3.702
		0.707			**0.355**	**0.057**	**0.178**	**0.011**	**0.030**	**1.002**	**0.381**	**0.059**	**0.098**
Hippocampus	NS-c	**3.972**			**0.162**	**0.117**		**0.775**		**4.246**	**5.815**	**1.406**	**5.814**
		0.254			0.023	0.012		0.094		0.300	0.126	0.087	0.264
	HYPER-G28	**4.056**			**0.132**	**0.109**		**1.968**		**4.553**	**5.255**	**1.160**	**5.926**
		0.126			0.033	0.015		1.148		0.267	0.337	0.056	0.309
Prefrontal cortex	NS-c	**4.573**			**2.664**	**0.748**	**0.491**	**0.323**	**0.185**	**11.164**	**7.673**	**0.697**	**5.669**
		0.439			0.471	0.064	0.088	0.046	0.019	0.603	0.319	0.040	0.173
	HYPER-G28	**4.464**			**3.029**	**0.753**	**0.451**	**0.342**	**0.153**	**10.676**	**5.700**[A]	**0.550**[A]	**5.968**
		0.098			0.672	0.055	0.126	0.076	0.023	0.665	0.308	0.052	0.291
Striatum	NS-c	**0.654**			**145.993**	**11.917**	**19.413**	**0.084**	**0.137**	**9.700**	**8.996**	**1.027**	**2.159**
		0.163			7.724	0.564	1.005	0.007	0.014	1.253	0.433	0.139	0.143
	HYPER-G28	**0.682**			**133.494**	**8.429**[A]	**19.271**	**0.064**[A]	**0.145**	**9.645**	**7.572**	**0.799**	**2.169**
		0.184			**3.772**	**0.307**	**0.966**	**0.004**	**0.009**	**1.109**	**0.616**	**0.028**	**0.118**
Nucleus accumbens	NS-c	**2.365**			**72.707**	**7.643**	**20.290**	**0.106**	**0.281**	**11.226**	**7.291**	**0.676**	**0.388**
		0.288			2.149	0.436	2.720	0.007	0.039	1.305	0.743	0.063	0.024
	HYPER-G28	**3.365**			**80.401**	**5.100**[A]	**16.702**	**0.065**[A]	**0.216**	**15.801**[A]	**6.043**	**0.404**[A]	**0.406**
		0.580			**4.501**	**0.779**	**1.937**	**0.011**	**0.033**	**1.448**	**0.217**	**0.041**	**0.018**

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
Locus coeruleus	NS-a	**24.776**	**5.445**	**0.225**	**3.448**	**0.967**	**2.339**	**0.302**	**0.690**	**17.921**	**10.408**	**0.600**	**0.364**
		2.452	0.509	0.018	0.438	0.064	0.269	0.031	0.033	2.277	1.089	0.039	0.030
	HYPER-G32	**33.839**[A]	**4.609**	**0.137**[A]	**3.679**	**0.825**	**2.388**	**0.229**	**0.648**	**18.147**	**9.478**	**0.550**	**0.360**
		1.992	0.312	0.007	0.269	0.077	0.197	0.026	0.017	1.913	0.602	0.050	0.022
Ventral bed nuc. stria terminalis	NS-a	**44.611**	**3.469**	**0.082**	**24.518**	**3.534**	**5.603**	**0.148**	**0.225**	**12.137**	**11.559**	**0.971**	**0.352**
		3.727	0.456	0.014	3.332	0.535	0.844	0.012	0.007	0.648	0.597	0.074	0.022
	HYPER-G32	**53.522**	**3.052**	**0.060**	**15.714**	**1.912**[A]	**3.994**	**0.142**	**0.249**	**11.364**	**11.007**	**1.037**	**0.371**
		3.309	0.584	0.014	3.174	0.318	0.907	0.036	0.012	1.189	0.936	0.115	0.026

Table 3 (*Continued*)

Area	Rat line	NE	MHPG	MHPG/NE	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT	Protein
Hypothalamus	NS-a	**20.121**	**1.411**	**0.071**	**4.209**	**0.417**	**0.697**	**0.101**	**0.166**	**13.805**	**11.266**	**0.832**	**4.550**
		0.906	0.076	0.005	0.209	0.042	0.097	0.012	0.022	0.814	0.537	0.059	0.123
	HYPER-G32	**24.232**[A]	**1.098**[A]	**0.046**[A]	**3.705**	**0.334**	**0.635**	**0.091**	**0.161**	**18.468**[A]	**12.653**[A]	**0.700**	**4.949**
		0.819	0.039	0.002	0.363	0.035	0.142	0.006	0.020	1.028	0.348	0.042	0.235
Hippocampus	NS-a	**4.352**	**1.361**	**0.317**	**0.215**	**0.150**	**0.169**	**0.848**	**0.809**	**4.340**	**6.624**	**1.556**	**4.853**
		0.194	0.029	0.016	0.037	0.010	0.046	0.146	0.197	0.251	0.423	0.127	0.137
	HYPER-G32	**4.579**	**1.147**[A]	**0.251**[A]	**0.184**	**0.155**	**0.149**	**0.936**	**0.871**	**4.511**	**6.304**	**1.406**	**5.065**
		0.116	0.041	0.010	0.023	0.018	0.063	0.175	0.400	0.154	0.239	0.064	0.193
Prefrontal cortex	NS-a	**4.313**	**1.773**	**0.414**	**2.318**	**0.764**	**0.499**	**0.337**	**0.219**	**11.208**	**5.066**	**0.456**	**6.753**
		0.176	0.064	0.015	0.165	0.042	0.024	0.022	0.010	0.483	0.395	0.036	0.316
	HYPER-G32	**4.930**[A]	**1.727**	**0.350**[A]	**2.220**	**0.638**[A]	**0.536**	**0.338**	**0.238**	**9.856**	**4.244**	**0.436**	**6.166**
		0.131	0.064	0.008	0.458	0.028	0.118	0.041	0.007	0.413	0.197	0.029	0.296
Striatum	NS-a	**0.777**	**0.612**	**0.782**	**144.940**	**11.457**	**19.091**	**0.079**	**0.131**	**11.337**	**8.820**	**0.790**	**2.379**
		0.043	0.073	0.076	5.819	0.561	1.425	0.004	0.006	0.610	0.439	0.052	0.113
	HYPER-G32	**0.893**	**0.474**	**0.552**[A]	**123.978**[A]	**8.573**[A]	**15.269**[A]	**0.070**[A]	**0.123**	**12.611**	**7.287**[A]	**0.600**[A]	**2.295**
		0.055	0.048	0.072	6.237	0.292	0.914	0.002	0.004	0.855	0.292	0.052	0.079
Nucleus accumbens	NS-a	**2.021**	**1.496**	**1.056**	**72.061**	**8.461**	**18.658**	**0.118**	**0.259**	**14.389**	**7.983**	**0.578**	**0.342**
		0.485	0.342	0.270	3.331	0.565	1.091	0.008	0.009	1.241	0.450	0.049	0.005
	HYPER-G32	**2.977**	**1.545**	**0.938**	**72.236**	**6.190**[A]	**16.985**	**0.086**[A]	**0.235**	**13.547**	**7.551**	**0.613**	**0.359**
		1.054	0.184	0.250	4.285	0.366	1.217	0.004	0.010	2.137	0.650	0.067	0.018

Concentrations shown are ng/mg protein. Means ± standard errors are shown. Group designations are: NS-a – non-selected, generation 38 of original line of non-selectively bred rats bred maintained with lines of selectively-bred rats [$n = 7$]; NS-c – non-selected, a different line of non-selected rats originated several generations after the original line of NS rats [$n = 7$]; HYPER-G28 – hyperactive rats, generation 28 of this line [$n = 8$]; HYPER-G32 – hyperactive rats, generation 32 of this line [$n = 8$]. **Statistical significance**: In each brain region and for each monoamine or metabolite in that brain region, a *t*-test was conducted comparing the HYPER group mean with NS group mean. A significant difference between the two groups (at least $p < .05$) is designated by the symbol "A" noted next to the mean of the HYPER group.

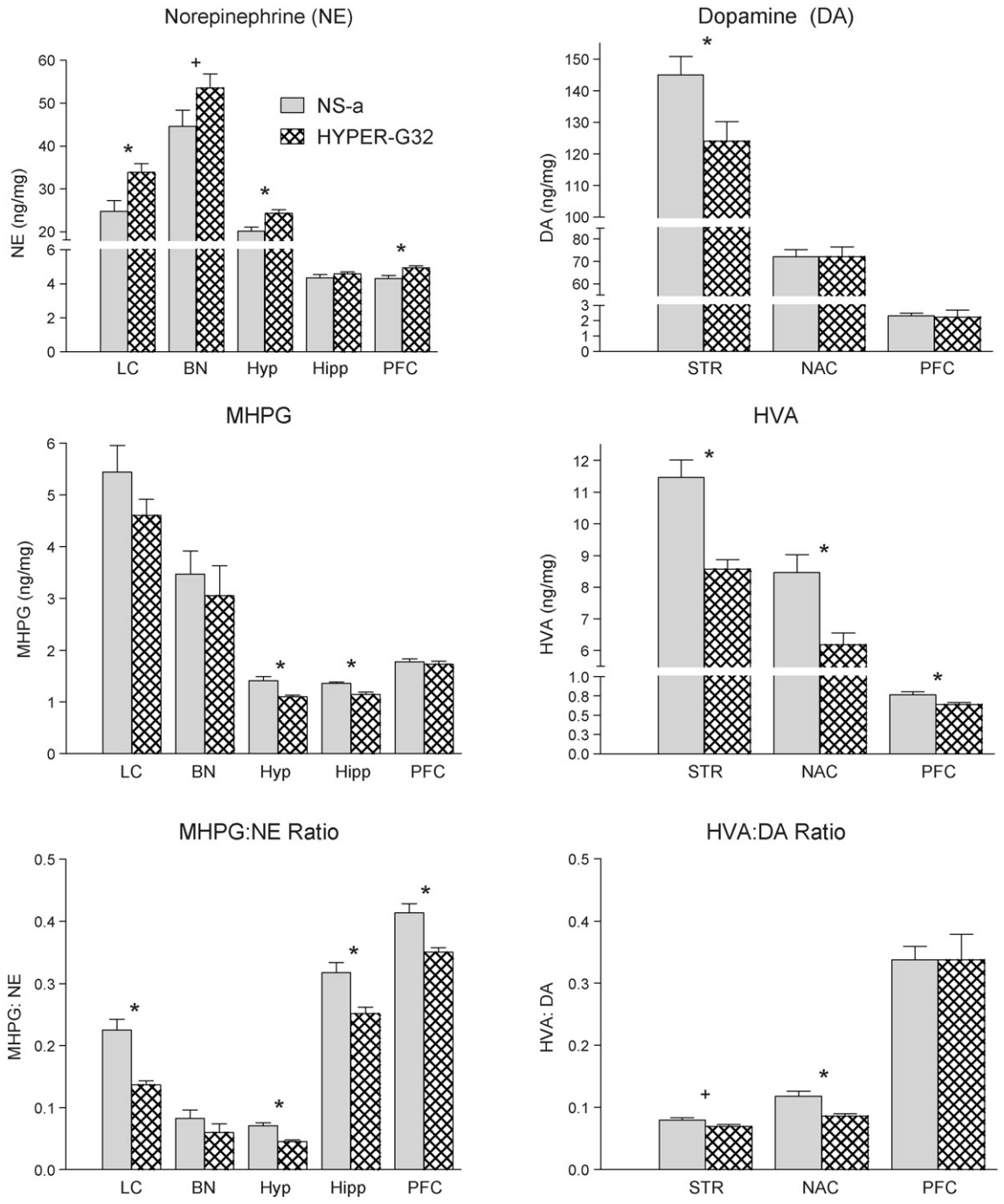

Fig. 11 – Concentration of norepinephrine (NE) and dopamine (DA), their respective principal extracellular metabolites (MHPG, HVA), and the ratio of the concentration of the metabolite to the concentration of the metabolite in various brain regions of Hyperactive rats from generation 32 (HYPER-G32) and non-selected (normal) rats that have been bred in parallel with the different lines of selectively-bred rats in the colony (NS-a). For monoamine and metabolites, values are ng/mg protein of the tissue sample. Brain regions: locus coeruleus (LC), ventral part of the bed nucleus of the stria terminalis (BN), hypothalamus (Hyp), hippocampus (Hipp), prefrontal cortex (PFC), striatum (STR), and nucleus accumbens (NAC). Means and standard errors are shown. (*) Significant difference ($p < .05$) between non-selected and hyperactive rats; (+) difference between non-selected and hyperactive rats approaches significance ($p < .10$).

reduced despite lower DA levels in the HYPER rats. The findings indicate that DA release in forebrain DA regions is lower in HYPER rats than in non-selected rats, particularly in the STR and NAC.

6.5. *Summary of neurochemical findings*

In different lines of rats that were selectively-bred to show different behavioral responses of potential relevance to the

study of affective disorders, measurement of brain monoamines (NE, DA, 5-HT) and their metabolites revealed distinct differences between the lines. These lines of rats were compared to three different lines of randomly-bred, non-selected rats (i.e., randomly bred or normal rats). In terms of the brain monoamine measurements made in this study, these non-selected lines were found to be quite similar to one another, and therefore can be said to represent an expected norm for the albino Sprague–Dawley laboratory rat, at least as assessed under the conditions present in our laboratory and by the methodology used in this study. Summarizing what was found, the selectively-bred lines differed in various respects from what was seen in the non-selected (i.e., normal) rats. Additionally, different lines showed interesting similarities to each other, but they also showed distinct differences as well, so that the different behavioral proclivities of each of the lines are represented by distinct neurochemical characteristics in regard to brain monoamines. Salient similarities and differences are described below.

First, both of selectively-bred lines that show reduced activity in the swim-test – SwLo rats "at baseline" and SUS rats in response to a stressor – showed markedly reduced concentrations, or levels, of NE in the hippocampus (shown in Fig. 9). What precisely this indicates in terms of function is not clear, particularly since the noradrenergic metabolite MHPG, which can be viewed as an indicator of NE release, was only slightly lower in these groups than in normal, non-selected rats. Nevertheless, the reduced NE level in the hippocampus of these lines was quite clear, and suggests that some aspect of NE function in this brain region is related to their vulnerability to showing reduced activity in the challenging situation of the swim test.

Second, markedly reduced levels of DA were seen in the STR of both HYPER and SUS rats. The HYPER rats also showed clearly reduced amounts of the dopaminergic extracellular metabolite HVA in STR. Reduced HVA in STR relative to non-selected rats was also seen in some generations of SUS rats, but this difference was less clear across all animals in the SUS line. Interestingly, the two lines (HYPER and SUS) show very different motor activity patterns – i.e., HYPER rats show nocturnal hyperactivity in the home cage while SUS respond to a stressor with markedly reduced activity in the swim test. Thus, reduced DA and DA release in the STR does not demarcate a single directionality for motor activity, but, rather, a tendency to show an exaggerated response to a stimulus (i.e., to the activity-inducing stimulus of "darkness" for the rat [in the HYPERS] and the activity-suppressive influence of stress [in the SUS rats]). Such findings suggest that dopaminergic activity in the STR exerts an important modulating, or moderating, effect on behavior, and this moderating influence is subnormal in both HYPER rats and SUS rats.

Third, regarding the differences that were seen between "alcohol drinkers" and "alcohol non-drinkers" in the SUS rats, the largest differences in monoamines that appeared between these groups were in the mesocorticolimbic DA regions (NAC and PFC that receive DA projections from VTA), with the differences pointing to reduced dopaminergic activity in NAC and PFC of alcohol drinkers as opposed to non-drinkers. Interestingly, the investigation of alcohol intake in SUS rats had been stimulated by some pilot data suggesting that 5-HT level was reduced in the brain of SUS rats. We then measured alcohol intake because reduced serotonergic activity had been linked to the tendency to drink alcohol (e.g., [60–62]). While our findings with respect to DA might be thought to indicate that, at least in SUS rats, any 5-HT differences are less important for alcohol consumption than are DA differences, reduced 5-HT level and a reduced amount of the serotonergic metabolite 5-HIAA were observed in the NAC of alcohol drinkers. Additionally, we have data (unpublished) showing that experimental elevation of serotonergic activity in SUS rats can counteract the proclivity of these animals to consume alcohol, thereby suggesting that brain serotonergic activity is involved in the alcohol-drinking tendency of SUS rats, and, perhaps, that the DA differences derive from, or at least coalesce with, reduced serotonergic activity in producing the tendency to consume alcohol that is seen in these rats.

Finally, while there were important instances where the concentration of an amine and its principle extracellular metabolite of that amine were both reduced or both elevated in a brain region (e.g., DA and its metabolite HVA in the STR of HYPER rats [described above]), there were several very prominent instances in which elevated levels of a monoamine were found together with markedly lower levels of the extracellular metabolite of that amine. Examples of this were: (a) in HYPER rats, a markedly higher level of NE in the LC compared with non-selected rats, but a much lower level of MHPG in the LC than in non-selected rats, and (b) in RES rats, highly elevated levels of DA in STR, NAC, and PFC compared with non-selected rats, but much lower levels of HVA in all three brain regions compared with non-selected rats. Such a finding – elevated level of the amine together with reduced indication of release – suggests the presence of marked feedback inhibition influencing the activity of the nerve terminals containing that amine in that brain region. This can occur as a consequence of a high level of stimulation of inhibitory receptors on nerve terminals which decreases release from the nerve terminals while allowing synthesis to produce accumulation of the monoamine in the terminal. Another possibility is highly responsive post-synaptic receptors for the monoamine in that brain region, resulting in feedback inhibition of release but allowing sufficient synthesis so that levels of the monoamine accumulate. This brings to the fore a most important point that needs to be considered when evaluating the neurochemical findings described in this paper. All of the findings presented in Tables 1–3, as well as in the illustrative figures showing data represented in these tables, describe what have been called "presynaptic" events, meaning events related to activity of the transmitter-containing and transmitter-releasing terminals. However, the actual effect of the transmitter on neural activity will depend on its interaction with receptors, either pre- or post-synaptic receptors, and no data are provided in this paper pertaining to the density, affinity, or types of receptors in the region. An example of the importance of such data for inferring activity relationships can be seen in SwHi and SwLo rats. In SwHi and SwLo rats, Table 1 shows that neither the level of DA nor of HVA in the NAC showed much difference in the two lines, but we found that that infusion into NAC of amphetamine, a dopamimetic agent that releases DA and stimulates DA receptors, had

markedly different effects on swim-test activity on SwHi and SwLo rats (West et al. [21]), thereby suggesting that there are large differences between SwHi and SwLo rats in receptor responsivity to DA in the NAC. Thus, with respect to describing neural activity as a result of monoaminergic actions in the brain regions assessed, the data presented here are incomplete, and any assumptions regarding this based on the data presented here should be made with considerable caution.

REFERENCES

[1] Weiss JM, Kilts CD. Animal models of depression and schizophrenia. In: Nemeroff CB, Schatzberg AF, editors. The American Psychiatric Press Textbook of Psychopharmacology. Washington, DC: American Psychiatric Press, Inc.; 1998. p. 89–131.

[2] Maier SF, Seligman MEP. Learned helplessness: theory and evidence. J Exp Psychol Gen 1976;105:3–46.

[3] Weiss JM, Bailey WH, Goodman PE, Hoffman LJ, Ambrose MJ, Salman S, et al. A model for neurochemical study of depression. In: Spiegelstein MY, Levy A, editors. Behavioral Models and the Analysis of Drug Action. Amsterdam: Elsevier; 1982. p. 195–223.

[4] Weiss JM. Stress-induced depression: critical neurochemical and electrophysiological changes. In: Madden IV J, editor. Neurobiology of Learning, Emotion and Affect. New York: Raven Press; 1991. p. 123–54.

[5] Englund SA, Klein DN. The genetics of neurotic-reactive depression: a reanalysis of Shapiro's (1970) twin study using diagnostic criteria. J Affect Dis 1990;18: 247–52.

[6] Gershon ES, Bunney Jr WE, Leckman JF, Van Eerdewegh M, DeBauche BA. The inheritance of affective disorders: a review of data and of hypotheses. Behav Genet 1976;6: 227–61.

[7] McGuffin P, Katz R, Rutherford J. Nature, nuture, and depression: a twin study. Psychol Med 1991;21:329–35.

[8] Weissman MM, Gershon ES, Kidd KK, Prusoff BA, Leckman JF, Dibble E, et al. Psychiatric disorders in the relatives of probands with affective disorders. Arch Gen Psychiatry 1984;41:13–21.

[9] Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386–9.

[10] Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. Arch Gen Psychiatry 2005;62:529–35.

[11] Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant procedures. Nature 1977;226:730–2.

[12] Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology 1988;94:147–60.

[13] Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M. Immobility induced by forced swimming in rats: Effects of agents which modify central catecholamine and serotonin activity. Eur J Pharmacol 1979;57:201–10.

[14] Satoh H, Mori J, Shimomura K, Ono T, Kikuchi H. Effect of zimeldine, a new antidepressant, on the forced swimming test in rats. Jpn J Pharmacol 1984;35:471–3.

[15] Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berlin) 1995;121:66–72.

[16] Weiss JM, Goodman PA, Losito BG, Corrigan S, Charry JM, Bailey WH. Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of the rat brain [review]. Brain Res Rev 1981;3: 167–205.

[17] Weiss JM, Cierpial MA, West CHK. Selective breeding of rats for high and low motor activity in a swim test: toward a new animal model of depression. Pharmacol Biochem Behav 1998;61:49–66.

[18] West CHK, Weiss JM. Effects of antidepressant drugs on rats bred for low activity in the swim test. Pharmacol Biochem Behav 1998;61:67–79.

[19] Van Praag HM. Psychotropic drugs: a guide for the practitioner. New York: Brunner/Mazel, Publishers; 1978.

[20] West CHK, Bonsall RW, Emery MS, Weiss JM. Rats selectively bred for high and low swim test activity show differential responses to dopaminergic drugs. Psychopharmacology (Berlin) 1999;146:241–51.

[21] West CHK, Boss-Williams KA, Weiss JM. Motor activation by amphetamine infusion into nucleus accumbens core and shell subregions of rats differentially sensitive to dopaminergic drugs. Behav Brain Res 1999;98:155–65.

[22] Scott PA, Cierpial MA, Kilts CD, Weiss JM. Susceptibility and resistance of rats to stress-induced decreases in swim-test activity: a selective breeding study.. Brain Res 1996;725:217–30.

[23] West CHK, Weiss JM. A selective test for antidepressant treatments using rats bred for stress-induced reduction of motor activity in the swim test. Psychopharmacology 2005;182:9–23.

[24] DSM-IV Task Force. Diagnostic and statistical manual of mental disorders: DSM-IV™. Washington, DC: American Psychiatric Association; 1994.

[25] Weiss JM, Boss-Williams KA, Moore JP, West CHK. Testing the hypothesis that locus coeruleus hyperactivity produces depression-related changes via galanin. Neuropeptides 2005;39:281–7.

[26] West CHK, Weiss JM. Intake of ethanol and reinforcing fluids in rats bred for susceptibility to stress. Alcohol 2006;38:13–27.

[27] Crabbe JC, Phillips TJ, Kosobud A, Belknap JK. Estimation of genetic correlation: interpretation of experiments using selectively bred and inbred animals. Alcohol Clin Exp Res 1990;14:141–51.

[28] Li T-K, Lumeng L, Doolittle DP. Selective breeding for alcohol preference and associated responses. Behav Genet 1993;23:163–70.

[29] Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, et al. Phenotypic and genotypic characterization of the Indian University rat lines selectively bred for high and low alcohol preference. Behav Genet 2002;32:363–88.

[30] Murphy JM, McBride WJ, Lumeng L, Li T-K. Contents of monoamines in forebrain regions of alcohol-preferring (P) and -nonpreferring (NP) lines of rats. Pharmacol Biochem Behav 1987;26:389–92.

[31] Gongwer MA, Murphy JM, McBride WJ, Lumeng L, Li T-K. Regional brain contents of serotonin, dopamine and their metabolites in the selectively bred high- and low-alcohol drinking lines of rats. Alcohol 1989;6:317–20.

[32] McBride WJ, Murphy JM, Lumeng L, Li T-K. Serotonin, dopamine and GABA involvement in alcohol drinking of selectively bred rats. Alcohol 1990;7:199–205.

[33] Lucas LAC, McMillen BA. Differences in brain area concentrations of dopamine and serotonin in Myers' high

ethanol preferring (mHEP) and outbred rats. J Neural Transm 2002;109:279–92.
[34] Morzorati SL. VTA dopamine neuron activity distinguishes alcohol-preferring (P) rats from Wistar rats. Alcohol Clin Exp Res 1998;22:854–7.
[35] Morzorati SL, Marunde RL. Comparison of VTA dopamine neuron activity in lines of rats selectively bred to prefer or avoid alcohol. Alcohol Clin Exp Res 2006;30:991–7.
[36] Fadda F, Mosca E, Colombo G, Gessa GL. Alcohol-preferring rats: Genetic sensitivity to alcohol-induced stimulation of dopamine metabolism. Physiol Behav 1990;47:727–9.
[37] Broadhurst PL. Experiments in psychogenetics. In: Eysenck HJ, editor. Experiments in personality: psychogenetics and psychopharmacology. London: Routledge and Kegan Paul; 1960. p. 3–102.
[38] Broadhurst PL. The Maudsley reactive and non-reactive strains of rats: a survey. Behav Genet 1975;5:299–319.
[39] Sudak HS, Maas JW. Behavioral–neurochemical correlation in reactive and non-reactive strains of rats. Science 1964;146:418–20.
[40] Slater J, Blizard DA, Pohorecky LA. Central and peripheral norepinephrine metabolism in rat strains selectively bred for differences in response to stress. Pharmac Biochem Behav 1977;6:511–20.
[41] Liang B, Blizard DA. Central and peripheral norepinephrine concentrations in rat strains selectively bred for differences in response to stress: confirmation and extension. Pharmac Biochem Behav 1978;8:75–80.
[42] Landgraf R, Wigger A, Holsboer F, Neumann ID. Rapid communication – hyper-reactive hypothalamo-pituitary-adrenocortical axis in rats bred for high anxiety-related behaviour. J Neuroendocrinol 1999;11:405–7.
[43] Keck ME, Sartori SB, Welt T, Müller MB, Ohl F, Holsboer F, et al. Differences in serotonergic neurotransmission between rats displaying high or low anxiety/depression-like behaviour: effects of chronic paroxetine treatment. J Neurochem 2005;92:1170–9.
[44] Kulikov A, Aguerre S, Berton O, Ramos A, Mormede P, Chaouloff F. Central serotonergic systems in the spontaneously hypertensive and Lewis rat strains that differ in the elevated plus-maze test of anxiety. J Pharmacol Exp Therapeut 1997;281:775–84.
[45] Seligman MEP. Depression and learned helplessness. In: Friedman RJ, Katz MM, editors. The psychology of depression: contemporary theory and research. Washington, DC: V.H. Winston; 1974. p. 83–125.
[46] Martin JV, Edwards E, Johnson JO, Henn FA. Monoamine receptors in an animal model of affective disorder. J Neurochem 1990;55:1142–8.
[47] Edwards E, Kornrich W, Van Houtten P, Henn FA. *In vitro* neurotransmitter release in an animal model of depression. Neurochem Int 1992;21:29–35.
[48] Overstreet DH, Russell RW, Crocker AD, Schiller GD. Selective breeding for differences in cholinergic function: pre- and post-synaptic mechanisms involved in sensitivity to the anticholinesterase, DFP. Brain Res 1984;294:327–32.
[49] Overstreet DH. The Flinders sensitive line rats: a genetic animal model of depression. Neurosci Biobehav Rev 1993;17:51–68.
[50] Zangen A, Overstreet DH, Yadid G. High serotonin and 5-hydroxyindoleacetic acid levels in limbic brain regions in a rat model of depression: normalization by chronic antidepressant treatment. J Neurochem 1997;69:2477–83.
[51] Zangen A, Overstreet DH, Yadid G. Increased catecholamine levels in specific brain regions of a rat model of depression: normalization by chronic antidepressant treatment. Brain Res 1999;824:243–50.
[52] Overstreet DH, Rezvani AH, Pucilowski O, Gause L, Janowsky DS. Rapid selection for serotonin-1A sensitivity in rats. Psychiatr Gen 1994;4:57–62.
[53] Knapp DJ, Sim-Selley LJ, Breese GR, Overstreet DH. Selective breeding of 5-HT_{1A} receptor-mediated responses: application to emotion and receptor action. Pharmacol Biochem Behav 2000;67:701–8.
[54] Overstreet DH, Rezvani AH, Knapp DJ, Crews FT, Janowsky DS. Further selection of rat lines differing in 5-HT-1A receptor sensitivity: behavioral and functional correlates [report]. Psychiatric Genet 1996;6:107–17.
[55] Gonzalez LE, Parada MA, Tucci S, Teneud L, Overstreet DH, Hernandez L. A brain microdialysis study on 5-HT release in freely moving rat lines selectively bred for differential 5-HT_{1A} receptor function. Braz J Med Biol Res 2003;36:263–7.
[56] Racine RJ, Steingart M, McIntyre DC. Development of kindling-prone and kindling-resistant rats: selective breeding and electrophysiological studies. Epilepsy Res 1999;35:183–95.
[57] McIntyre DC, Kent P, Hayley S, Merali Z, Anisman H. Influence of psychogenic and neurogenic stressors on neuroendocrine and central monoamine activity in fast and slow kindling rats. Brain Res 1999;840:65–74.
[58] Anisman H, Kelly O, Hayley S, Borowski T, Merali Z, McIntyre DC. Acoustic startle and fear-potentiated startle in rats selectively bred for fast and slow kindling rates: relation to monoamine activity. Eur J Neurosci 2002;12:4405–16.
[59] Murrough JW, Boss-Williams KA, Emery MS, Bonsall RW, Weiss JM. Depletion of brain norepinephrine does not reduce spontaneous ambulatory activity of rats in the home cage.. Brain Res 2000;883:125–30.
[60] LeMarquand D, Pihl RO, Benkelfat C. Serotonin and alcohol intake, abuse, and dependence: findings of animal studies. Biol Psychiatry 1994;36:395–421.
[61] McBride WJ, Murphy JM, Gatto GJ, Levy AD, Lumeng L, Li TK. Serotonin and dopamine systems regulating alcohol intake. Alcohol Alcohol 1991;1:411–6.
[62] Overstreet DH, Halakis JA, Seredinin SB, Kampov-Polevoy AB, Viglinskaya IV, Kashevskaya O. Behavioral similarities and differences among alcohol preferring and nonpreferring rats: confirmation by factor analysis and extension to additional groups. Alcohol Clin Exp Res 1997;21:840–8.