Behavioral and Biochemical Issues in Substance Abuse

Frank R. George, PhD
Doris Clouet, PhD
Guest Editors

Barry Stimmel, MD
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Behavioral and Biochemical Issues in Substance Abuse

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EDITORIAL

Behavioral and Biochemical Genetic Issues in Substance Abuse

For many years there has been significant interest and research within the area of biological vulnerability to alcohol abuse. However, with other abused substances, such as opiates and cocaine, historically the majority of interest and research effort has dealt with environmental and behavioral variables which contribute to substance abuse disorders. Due in part to the clear contribution of genetic factors in alcohol abuse, and data indicating genetic differences in response to many drugs in animal models of substance abuse and drug effects, the National Institute on Drug Abuse (NIDA) has increased its interest in the contribution of genetic factors and biological vulnerability to substance abuse. One result of this increased interest was the decision to organize a NIDA Technical Review conference entitled "Behavioral and Biochemical Genetic Issues in Substance Abuse." This conference was held June 16-17, 1989 at Keystone, Colorado USA, in conjunction with the annual meeting of the Committee on Problems of Drug Dependence, Inc. This meeting served several purposes: (1) it brought together investigators currently working on the genet-

ics of substance abuse; (2) it helped to define the state of knowledge in this area; (3) current needs and future directions were discussed; and (4) it promoted NIDA's increasing interest in this area.

A broad range of interests were represented at this meeting, as the participants included NIDA administrative officers, as well as university and government researchers conducting studies involving humans, rodents and cell cultures. In addition, a number of individuals, representing a broad range of interests in substance abuse issues, attended the conference and contributed to the discussion sessions. The following papers which make up this issue of *Journal of Addictive Diseases* are based upon the presentations from this conference. Regardless of the specific methods and species used, there are some common themes and concepts which unite these papers as well as the investigators as behavioral geneticists.

Perhaps the primary concept which underlies this area is that genes can be used as independent variables. This idea forms the basis for a unique and powerful approach to biomedical research in general and to drug abuse research in particular. The understanding that genes can be used as independent variables is important for two critical reasons. The first reason is that genotype can be systematically manipulated or varied, which allows for the estimation of genetic contributions to various dependent measures, such as behavioral responses to drugs. The second reason is that genotype can be controlled or held constant. When genotype is held constant, then any variation found in a dependent measure is presumably due to environmental effects. Therefore, the ideal way to study genetic factors is to thoroughly control for environmental variation, and the ideal way to study environmental factors is to completely control for genetic variation.

One overall consensus reached at this conference was that, in terms of non-alcohol related substance abuse studies, environmental effects have been explored and controlled for in an excellent manner, but genetic factors are very poorly understood and controlled, and that this is an area where further progress is important to our understanding of both genetic as well as environmental factors involved in substance abuse.

Genetic variation in response to drugs can be used as a powerful research tool. In terms of animal models, genetic selection for divergent phenotypes related to drug effects is an important method which is being effectively used in studying alcohol, and is now being adopted into a growing number of projects related to mechanisms of non-alcohol drugs, such as opiates, nicotine, cocaine and benzodiazepines. The paper by Andrew Smolen and Michael Marks[1] describes the use of selective breeding

paradigms to produce novel lines of mice which differ maximally in Y-maze activating response to nicotine, and additional independent lines of mice which differ in activating response to cocaine. Initial findings show clear evidence of heritability for these responses, and early progress is encouraging. John Belknap[2] presented elegant autoradiographic data related to his selective breeding project for high vs. low antinociceptive response to levorphanol. His mice currently differ by seven-fold in this measure of analgesia, and he showed that these mice show significant differences in density of dorsal raphe nucleus mu receptor sites. Edward Gallaher and coworkers[3] have developed selectively bred lines of mice which differ substantially in response to diazepam when using rotorod stability as the phenotype. Interestingly, these mice do not differ in diazepam-induced seizure protection, suggesting that the anticonvulsant and muscle relaxant effects of diazepam are associated with different biological pathways. The paper by Janice Froehlich and Ting-Kai Li[4] reviews the status of the widely recognized alcohol-preferring and alcohol non-preferring rat lines. Studying the procedures used in their successful alcohol studies can aid in the development of appropriate selective breeding studies for divergent intake of non-alcohol drugs of abuse.

Another method which holds great potential in the study of substance abuse involves the use of Recombinant Inbred (RI) strains of rodents. The report by Tamara Phillips and coworkers[5] provides an excellent introduction to the use of RI strains in identifying major gene effects, mapping traits to particular chromosomes, and in studying genetic relationships among different traits. The authors then describe the status of their own work using these methods in studying possible major gene effects moderating alcohol or morphine intake. Jeanne Wehner and coworkers[6] discuss the use of RI strains as it relates to the development of the LSXSS RI strains, which have recently been derived from the well known LS and SS selectively bred lines. These authors show how these LSXSS RI mice have already been used to estimate the number of genes involved in mediating neurosensitivity to ethanol, and how they are being used to study biological mechanisms associated with responses to ethanol.

A third pharmacogenetic method used by several of the participating investigators involves comparing responses on various behavioral and/or biochemical phenotypes across multiple inbred strains to obtain an estimate of the relationship, or correlation, among the traits under study. For example, the report by Allan Collins and Michael Marks[7] describes their studies examining the relationships among several behavioral and physiological responses to nicotine with numbers of brain nicotinic receptors in

various brain regions. These authors also present preliminary findings suggesting the presence of genetic differences in nicotine intake in mice.

This idea of genetic differences in drug-taking behaviors is being studied in a growing number of laboratories. The report by Frank George[8] summarizes the work by him and his associates on genetic contributions to alcohol, opiate and cocaine self-administration and home cage drinking. The findings are intriguing in that they suggest possible common determinants of reinforcement from these different abused substances. These studies are complemented by the work presented in the two papers by John Carney, Thomas Seale and their colleagues.[9,10] These investigators present pharmacogenetic data on a number of animal models of response to cocaine as well as possible reinforcement from cocaine, using nonself-administration models such as conditioned place preference. Taken together, these three reports[8-10] present an important direction in the field as they will hopefully result in a better defined and greater consensus on what is meant by "drug reinforced behavior".

Across all of the behavioral studies it is important to keep in mind the specific phenotypes being measured, and their specific advantages and disadvantages. The paper by Richard Meisch[11] addresses methodological issues in two behavioral areas critical to our understanding of substance abuse behavior, namely drug-reinforced behavior and drug discrimination. For example, it is important to distinguish between the study of initiation of self-administration or discrimination and the maintenance of these behaviors, as different methods will be involved, and the behaviors may likely be mediated by dissimilar mechanisms.

Much of the work in the reports at this meeting has as one ultimate goal defining those underlying biochemical traits associated with behavioral responses to drugs, especially drug self-administration. Mary Ritz[12] presents findings as well as important methodological and conceptual concerns relevant to the conduct of biochemical genetic studies. One important question raised is how much effect does one gene have on a receptor. We know that there are a number of significant single gene effects on behavior, but how do single genetic variants affect receptor structure or regulation? For example, as much as the dopamine transporter site appears to be highly associated with the reinforcing effects of cocaine, she shows that there are no significant differences in ligand affinity or number for either the dopamine transporter site or D1 or D2 receptor sites across rat strains that differ in self-administration of cocaine.

At this meeting the status and future of human genetic studies on substance abuse were also discussed. Roy Pickens and Dace Svikis present

reviews of findings from human adoption and twin studies of substance abuse.[13,14] Dr. Pickens stresses the complementary nature of human and animal model studies arguing effectively for the importance of both levels of investigation.[13] Dr. Svikis examines several methodological assumptions and procedures important in the appropriate conduct of human genetic studies in substance abuse.[14]

A specific issue in human studies discussed at the meeting is specific population prevalence, an issue raised by both Drs. Pickens and Svikis in their presentations. For example, when studying twins reared apart, one twin may be reared in a region with high prevalence of cocaine use while the other twin is reared in a region with low cocaine use. Under these conditions, it may be difficult to assess whether non-concordance for cocaine abuse is the result of a lack of genetic influence on this trait, or due to confounding environmental factors such as drug access.

An exciting possibility in substance abuse research which is just beginning to emerge is the use of cell culture studies as presented in the work by John Madden and Arthur Falek.[15] These authors show that nonneuronal cells can react directly with opiates *in vitro*, which has significant impact on metabolic processes within these cells. This work may lead to studies with twins or families which would involve not just psychosocial or behavioral measures, but also the attainment of peripheral cell populations that can be cultured and studied in terms of specific receptor populations. This would provide a method for determining markers and risk for substance abuse disorders.

Together, the findings from this meeting, whether based upon human, rodent or cellular studies, indicate that there are large genetic differences in response to abused substances. Animal models are being effectively utilized to examine drug mechanisms and correlated traits, while at the human level emerging findings indicate a need for further studies to identify specific biological factors which contribute to individual variation in responses to drugs and in the development of substance abuse disorders.

We sincerely thank all of the participants for contributing to an exciting, interesting and informative meeting. It is hoped that this meeting and these papers will contribute significantly towards a greater understanding of the mechanisms of action of abused substances, and will aid in determining the extent of biological vulnerability and risk for the development of substance abuse disorders in humans.

Frank R. George, PhD
Doris Clouet, PhD

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Genetic Selections for Nicotine and Cocaine Sensitivity in Mice

Andrew Smolen, PhD
Michael J. Marks, PhD

SUMMARY. We are using selective breeding to develop lines of mice which differ maximally in their responses to nicotine, and independent lines of mice which differ maximally in their responses to cocaine. The foundation population was the genetically heterogeneous HS mice. On day 1, baseline (saline injected) activity of each mouse was measured in an automated Y-maze over 3 minutes. On day 2, animals were tested for sensitivity to nicotine (0.75 mg/kg) in the same apparatus. A residual score, calculated from the regression of nicotine scores on saline scores for the whole population, was calculated for each animal. The most severely affected mice (lowest residual scores) were mated to form duplicate Nicotine-Depressed lines; the most stimulated mice (highest residual scores) were mated to form duplicate Nicotine-Activated lines. A random sampling of individuals was chosen without regard to residual scores for production of duplicate Control lines. Duplicate lines of mice activated and depressed by cocaine are being produced in an analogous fashion using 50 mg/kg cocaine as the test dose. Successful selective breeding for a drug-related trait provides clear evidence of a heritable component for that trait. These selected lines of mice will ultimately be used to study hypotheses involving genetic control of response to these drugs.

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The role of genetic factors in influencing drug responses in animals can be studied using inbred strains, recombinant inbred strains, heterogeneous populations or lines of animals genetically selected to differ maximally for a trait of interest.[1] Each of these approaches has been used to some extent, but the overwhelming majority of genetic investigations has concentrated on inbred strain comparisons. We are in the process of developing lines of mice which are differentially sensitive to nicotine, and independent lines which are differentially sensitive to cocaine. In this paper we will discuss some of the advantages of using selective breeding for differential drug sensitivity, outline some of the considerations which should be addressed in designing and implementing such a study, and provide a brief description of the results obtained from testing of the foundation population.

Inbred strains, which have been most widely used in genetic studies, are produced by mating close relatives, generally brother-sister, over numerous (20 minimum) generations to obtain fixation and homozygosity of virtually all genetic loci. One member of an inbred strain can be considered to be a genetic replicate of all other members of that strain. The major advantage of inbred strains is their relative constancy over time. Members of an inbred strain tested today for a particular biochemical or behavioral trait are very nearly the same as members of that strain tested several years ago on the same measurement. If several inbred strains reared in the same laboratory are found to differ in response to a drug, this is taken as *prima facie* evidence for a genetic basis for the difference. A second advantage of using inbred strains for genetic studies is the availability of literally hundreds of strains. A screen of a modest number of these strains can often reveal two which differ markedly in a trait of interest. These strains can then be used to investigate potential genetic regulation of the trait of interest using methods such as classical cross (comparison of 2 inbred strains, their F_1 hybrids, and F_2 and backcross generations) and diallel (comparison of several inbred strains and all possible F_1 crosses) analyses. Thirdly, inbred strains are often well characterized for a number of biochemical and behavioral traits. This can be of advantage in the initial choice of strains to screen or to assess potential confounding characteristics of a strain.

The genetic homogeneity of inbred strains, which may be advantageous when comparing animals across time and laboratories, is a marked disadvantage when one is interested in differences between individuals or correlations between two or more responses. The total phenotypic variance of a population, V_P, can be described as the sum of its genotypic variance, V_G, its environmental variance, V_E, and variance due to possible interac-

tions between genotype and environment, $V_{(G \times E)}$: $V_P = V_G + V_E + V_{(G \times E)}$.[2,3] Since fixation of alleles reduces genetic variance within strains to nearly zero, inbred strains are of limited value in studies where correlational analysis is to be employed. Two traits may correlate highly among inbred strains because of chance fixation of alleles during inbreeding, and may not imply a cause-effect relationship.

Recombinant Inbred (RI) strains offer a more sophisticated approach to studying genetic mechanisms underlying differences between the two inbreds. RI strains are produced by mating 2 inbred strains to produce the F_1 generation. The F_1 animals are then mated to produce the F_2 generation. This is a segregating population in which individuals may have different alleles at any locus where the original inbred strains differed. The purpose of the F_2 generation in producing RI strains is to shuffle the alleles of the parental strains randomly among individuals and to break up linkages between loci. Pairs of male and female F_2 siblings are then chosen to produce multiple (40 or more) RI strains, which are considered inbred after 20 generations of full-sib matings. The resulting RI lines contain random pairings of the genetic information at each locus from the parental lines. The major use of RI strains is to test for single gene effects using strain distribution patterns as described in another paper in this volume. The major disadvantage to RI strains is that the genetic information they provide is limited by the degree to which the parental strains differed. They are, however, powerful genetic tools which have been underused for studies of drug effects.

Heterogeneous populations offer another method for studying genetic effects on drug responses. In this case the genotypic variance of the population is non-zero, and differences among individuals is due to genetic as well as environmental factors. A genetically heterogeneous population is of much more utility in correlational studies than are inbred strains. In this case the contribution of the genotypic co-variance to the correlation is much less likely to be fortuitous; thus, one may undertake to investigate genetic mechanisms underlying correlations between two measurements.

There are comparatively few heterogeneous stocks of laboratory animals for use in pharmacogenetic research. One is often limited to lines such as Swiss-Webster mice or Wistar or Sprague-Dawley rats. These animals are generally regarded as being "outbred" and therefore heterogeneous to some extent, but in most cases their history is simply not known. Depending on the source, these lines may be considerably inbred, which is also indicated by the fact that all of these lines are albino (a recessive allele). In contrast, McClearn and coworkers[4] constructed a het-

erogeneous stock of mice (HS) by intercrossing eight inbred strains. The HS stock is maintained by randomly mating 40 pairs of animals with no common grandparental ancestry each generation. These animals represent a truly heterogeneous population and have been used for a wide range of studies; however, their main utility has been as the foundation population for a number of genetic selection studies.

The use of a heterogeneous foundation population for selecting lines of animals with large behavioral differences was first demonstrated by Tolman[5] in his studies of maze learning in rats. Tolman's two-generation selection study formed the foundation for the classical work of Tryon[6] who succeeded in establishing maze-bright and maze-dull lines of rats with non-overlapping distributions after only seven generations of selection. This well-known selection experiment was in turn a prototype for a number of other selection experiments for such diverse characteristics as motor activity[7] and emotionality in rats,[8] and body weight,[9] susceptibility to audiogenic seizures,[10] litter size and lactation,[11] open-field activity,[12] acoustic priming-induced seizures[13] and nest building[14] in mice. The utility of selective breeding in establishing phenotypes for the pharmacological investigations of drug responses is exemplified by selection studies for ethanol preference in rats,[15,16] acute response to ethanol,[17] severity of the ethanol withdrawal syndrome,[18,19] hypothermic effects of ethanol,[20] differential ethanol-induced locomotor activity,[21] sensitivity to diazepam[22] and sensitivity to opiate antinociception[23] in mice.

A successful selective breeding program for a drug-related phenotype provides clear evidence of a heritable component for that phenotype, and the resulting animals may be very useful for the study of the mechanisms through which the genes exert their influence upon the phenotype. In order for a selection experiment to be successful, there must be recognizable individual differences within the population. Some of this variation is due to environmental factors, but this source of variation is not heritable and does not influence response to selection pressure. In a properly designed selection experiment, differences among lines will be due at least in part to changes in frequencies of alleles which determine, directly or indirectly, that phenotype. Thus, a correlated response to selection implies a genetic correlation with the selected character. Selected lines are, therefore, effective for testing hypotheses concerning drug actions, since differences between the lines, be they behavioral (e.g., drug self administrarion), physiological (e.g., effects on heart rate) or biochemical (e.g., regulation of neurotransmitter receptors), are likely to be related to the mechanism of action of the drug. For example, if the activity of a neuro-

transmitter system thought to be involved in drug sensitivity is found to be the same in both lines, that system probably does not mediate that response.

Another advantage of a selection study is that the response of the selected lines to the selection criterion often exceeds the maximum differences in the foundation population. This has been well demonstrated by the long-sleep (LS)/short-sleep (SS) mouse selection by McClearn and Kakihana,[17] and the selection for open-field activity in mice by DeFries and coworkers.[12,24,25] Both studies resulted in selected lines with means which far exceeded the range of responses in foundation population. Thus, by changing allelic frequencies by selective breeding, it is possible to produce animals with responses exceeding those of natural populations. This can be utilized to great advantage when testing hypotheses concerning drug action.

In this paper we will outline our approach to using selective breeding to develop lines of mice which differ maximally in their responses to nicotine, and independent lines of mice which differ maximally in their responses to cocaine. The availability of these selectively bred lines will enable us to examine hypotheses involving genetic control of responses to these drugs.

METHODS

The goal of a bi-directional selection study is to accumulate alleles involved in sensitivity to a drug in the sensitive lines and alleles involved in drug resistance in the resistant lines while leaving all other alleles randomly distributed. A properly designed genetic selection experiment must include certain features at its inception to insure that possible chance associations between spurious parameters and the selected phenotype are kept to a minimum.[24-28] Since response to selection is a function of the amount of additive genetic variance present in a population, it is important to maintain genetic variance within the selected lines. This may be readily accomplished by starting with a population as heterogeneous as possible, and by using as large a number of mating pairs per line as feasible to insure that inbreeding within the selected line is kept to a minimum. In a randomly mated population of mice, a closed line consisting of 10 mating pairs will have a coefficient of inbreeding of less than 1.5% per generation,[2] which is generally considered to be acceptable.

A second important consideration in a selection experiment is the inclusion of a contemporaneous, unselected control line containing a number of mating pairs equal to the selected lines. A control line is useful to evaluate

effects of possible intergeneration environmental fluctuations and effects of possible inbreeding in the selected lines. The high and low lines may each be measured by their deviation from the control mean; thus, any asymmetry of response to selection (either direct or correlated) may be ascertained.

A third critical feature which must be included in a selection study is replication. Since large intergeneration variability is often found in a selection study, especially early on, replicated lines allow for the assessment of the generality of response to selection. Replication is especially important to test hypotheses concerning mechanisms. Chance changes in frequencies of alleles unrelated to the character under selection will often occur. If replicates of the lines are available, any hypothesis of genetic association may be tested immediately. Since chance associations between characters unrelated to the phenotype under selection would not be expected to occur in both replicates, a correlated response found in both replicates is likely to be due to causal factors.

A fourth feature which should be included in the design of a selection experiment is bi-directional selection: the contemporaneous selection of lines more and less sensitive than the foundation population. Selecting high and low lines simultaneously maximizes the potential to produce large differences between the lines. This also requires that the test used allows for scores which can go higher or lower than those in the foundation population.

Our selection studies contain each of the features listed above. The foundation population was the genetically heterogeneous HS mice. Genetic heterogeneity is being retained by maintaining 10 mating pairs for each selected line. We are producing replicated lines of mice which show (1) reduced (depressed) locomotor activity (compared to saline baseline) following nicotine administration (Nicotine Depressed "ND1," "ND2"); (2) increased (activated) locomotor activity following nicotine administration (Nicotine Activated, "NA1," "NA2"); (3) reduced locomotor activity following cocaine administration (Cocaine Depressed "CD1," "CD2"); and (4) increased locomotor activity following cocaine administration (Cocaine Activated, "CA1," "CA2"). Moreover, replicated unselected control lines ("C1," "C2") are being produced. One advantage of performing the selection studies for nicotine and cocaine simultaneously is that the same replicate control lines can be used for both selection studies, thus saving animals and space. We are performing bidirectional selection for each drug using a locomotor test that allows for both increased and decreased activity. Finally, we are employing within-

family selection which minimizes inbreeding. For example, in the resistant lines, the most resistant male and female from each of 10 litters are selected for mating. These animals will be mated randomly to produce the next generation.

Experimental Animals

The foundation population for the selection studies was the Heterogeneous Stock mice developed by McClearn and coworkers.[4] The selected lines were derived from 40 families of HS mice currently on hand. All mice are housed in the Specific Pathogen Free (SPF) facility of the Institute for Behavioral Genetics (IBG). Mice are kept in a constant temperature, constant humidity environment with a 12-hr light cycle (lights on 0700-1900). and are allowed free access to food (Wayne Sterilizable Lab Blox) and water.

Drugs

The drugs we are studying are nicotine and cocaine. Concurrent selection studies on these drugs will allow us to study potential commonalties between nicotine and cocaine directly. It has been suggested that both drugs (and amphetamine, as well) exert at least some of their effects by causing the release of, or inhibition of, reuptake of catechol- and indoleamines, either of which would result in increased synaptic concentrations of biogenic amines. The possibility that cross-tolerance (or sensitivity) might occur between them is suggested by studies which have shown that smokers are often unable to distinguish between intravenously administered cocaine or nicotine.[29]

Y-Maze Activity

The Y-maze measures voluntary locomotor activity. The apparatus is an enclosed red Plexiglas Y-maze 25 cm × 25 cm × 10 cm, divided into six areas (two per arm) by photoelectric beams. Crossing of a beam activates a counter which accumulates the number of beams crossed during the 3-min. test. The number of beams crossed is recorded as the total activity score. An additional set of photoelectric beams mounted 5 cm above the floor of the apparatus is used to count the number of rearings. Number of rearings is measured as a correlated response to selection. Testing is conducted between 1000 and 1500 hours.

Body Temperature

The assessment of the effects of drugs on body temperature is a relatively easy and reliable test in a constant temperature environment such as the SPF laboratory at IBG. Rectal temperature is measured using a Thermalert THS probe (Bailey Instruments). This probe equilibrates within 5 sec. and measures temperature to the nearest 0.1°. Body temperature is being measured as a correlated response to selection.

Regression Residuals as the Selection Criterion

Since we are interested in drug-induced activity, the simplest method would be to administer the drug to the mouse, measure its activity level, and then choose the most active for the high lines and the least active for the low lines. That system, however, does not take into account the animal's baseline (saline-injected) activity level, and it is possible that we would be selecting on the basis of overall, not specifically drug-induced, activity. In order to select for drug induced response, some method of controlling for basal activity should be used. Difference scores (drug minus saline) have commonly been used for this purpose. There are, however, a number of problems with difference scores, the most serious being that they are often found to be negatively correlated with baseline measurements.[30] In contrast, deviations from regression, more commonly called regression residuals, correct for any correlation between pretreatment and posttreatment measurements.[30,31] Regression residuals represent the difference between drug response predicted from the saline vs. drug regression line and the actual response measured.

Comparisons between difference scores and regression residual scores can best be seen with a simple, model data set as shown in Table 1. The regression equation is calculated for the whole population with observed (measured) saline score (S_i, column 1) on the x-axis and observed (measured) drug score (D_i, column 2) on the y-axis. For this example, expected drug score = 2 + [0.2 * (saline score)]. Each animal's saline score is substituted into the equation, and an expected drug score ($\hat{D}_i$) is calculated for each individual (column 3). The regression residual for that individual (denoted $D_i - \hat{D}_i$) is simply the difference between the observed drug score and the expected drug score (column 4). These data are graphically represented in Figure 1. The difference score ($D_i - S_i$) for each individual is tabulated in column 5. Some properties of difference scores and regression residuals are shown by the correlations listed at the bottom of Table 1.

The correlation coefficient, r, and the regression coefficient, b (the slope of the regression line), are both 0.2 for this example (equation 1).

TABLE 1. Comparison of Deviations from Regression with Difference Scores

Saline Score (observed) S_i	Drug Score (observed) D_i	Drug Score (expected) $\hat{D}_i$	Regession Deviation Score $D_i - \hat{D}_i$	Difference Score $D_i - S_i$
3	1	2.6	-1.6	-2
4	4	2.8	+1.2	0
5	5	3.0	+2.0	0
6	2	3.2	-1.2	-4
7	3	3.4	-0.4	-4

(1) Correlation and regression coefficients obtained from the plot of saline scores vs. drug scores:

$$b_{DS} = 0.2, \qquad r_{DS} = 0.2$$

(2) Regression equation obtained from the plot of saline scores vs. drug scores:

$$\hat{D}_i = \bar{D} + b_{DS} (S_i - \bar{S}) = 2 + 0.2 S_i$$

(3) Correlation between difference scores and saline scores:

$$r_{(D_i - S_i)(S_i)} = -0.63$$

(4) Correlation between residual scores and saline scores:

$$r_{(D_i - \hat{D}_i)(S_i)} = 0$$

(5) Correlation between difference scores and drug scores:

$$r_{(D_i - S_i)(D_i)} = 0.63$$

(6) Correlation between residual scores and drug scores:

$$r_{(D_i - \hat{D}_i)(D_i)} = 0.98$$