Despite the earlier report[2] to the contrary the increases in cathepsin B and D (fig. a), together with other lysosomal enzymes[10], in the functionally overloaded soleus would appear to support the view that protein degradation is accelerated[5]. Hence this form of compensatory growth involves an increase in both the protein synthetic and degradative rates[5]. However, since the former rate exceeds the latter, the net effect must be growth. The similarity of these changes in both functionally overloaded and immobilized (stretched[11]) muscles serves to strengthen the concept that passive stretch may be a common factor promoting growth in both experimental systems[5,12].

Previous studies[5,13] have also suggested that the newly synthesized proteins in the overloaded soleus are of a more 'labile' nature and are therefore more susceptible to degradation when this tissue is suddenly rendered inactive e.g. after nerve section. Denervation alone certainly causes cathepsin B and D activities to increase (fig. a). This is in agreement with other earlier studies[7] and previously reported increases in protein breakdown[14]. However, when inactivity was imposed upon the formerly overloaded muscle by denervation a roughly additive increase in proteolysis was observed (fig. a). Once again this supports the earlier observations of an additional increase in protein degradation[5], and possible greater lability of proteins[5,13], when this form of compensatory growth is arrested by denervation.

Denervation alone also increased the proteinase activities in the gastrocnemius (fig. b). However no additive effect was found in this muscle when tenotomy and denervation were combined. The latter is not particularly surprising since, unlike the overloaded soleus, there is less reason to predict any change in the lability of the proteins still being synthesized in a wasting tenotomized muscle.

Throughout the above studies the induced alterations in cathepsins B and D consistently changed in parallel with reported changes in the rates of protein breakdown under identical experimental situations[5]. On the basis of these (fig.), and our earlier[6], findings a good correlation appears to exist in skeletal muscle between these two endopeptidases and protein degradative rates. Interestingly here (fig.), as in previous studies[6], cathepsin H activity appeared very resistant to change. It is not immediately obvious why this should be, especially since all three proteinases are probably lysosomal in origin. The failure of this enzyme to respond in like manner may not become apparent until we obtain a better understanding of the mechanical and chemical events which link the changes in muscle activity and protein turnover.

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Inbreeding considerations in a REM sleep model for rat swimming activity

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Summary. Over the past few years, our laboratory group has elaborated a repeated measures rat swimming test. It provides an animal base for showing that the REM sleep mechanism is important to both emotional responsiveness and environmental adaptations. All of that work has been done with Sprague-Dawley rats obtained from a local supplier. Work done with two European rat stocks (by researchers in France and The Netherlands) shows general agreement with our own. In this presentation, we directly compare rats derived from an English vendor's Sprague-Dawley stock with the U.S. based Sprague-Dawley stock which we have been using. We also make strain comparisons via the F344 and the Long Evans strains. Although the literature has numerous examples of swimming test differences between inbred and wild rat stocks, strain difference effects have not been reported. We report that there are significant differences attributable to inbred strain but not to vendor on this measure.
Key words. Adaptation; animal models; animal vendor effects; evolutionary mechanisms; rat strain effects; rat swimming; sleep.

We have previously presented a serial, week-long, procedure known as the rat swimming test[2]. The present report is based on the key outcome from that protocol: After an initial period of vigorous swimming activity, rats in a swimming cylinder gradually learn to adopt a characteristic posture. An experienced rat simply lies quietly with only its eyes and nose unsubmerged in the water. This postural stillness is known as swimming immobility[3,4]. Since it conserves energy, we consider this behavioral change to be an adaptive response to a stressful situation[4]. Of theoretical interest is the finding that rats which have been REM sleep deprived, do not show improvement with regard to such immobility[2]. Instead, REM sleep deprived rats become progressively more active with succeding days of REM sleep deprivation treatment. The effect is reversable. Just 24 h after undisturbed sleep is allowed, the REM sleep deprived animals also show a high level of the immobility response. It is important to note that the procedure does not affect all of an animal's behavioral repertoire. Two concomitant measures (diving and headshaking) show no changes with REM sleep deprivation treatment. We conclude, therefore, that rat swimming immobility is a behavior which is selectively affected by REM sleep deprivation[2].

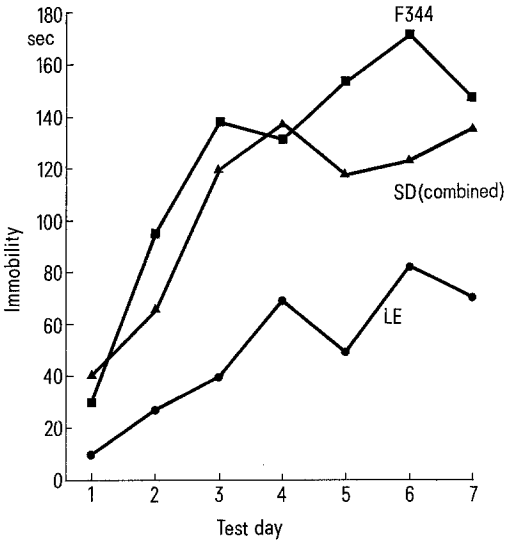
Long term REM status is important to such adaptive responsiveness. We infer this from ontogenetic work involving environmental enrichment[5,6]. A number of polygraphic studies have compared rats or mice which had been raised in enriched as compared with impoverished laboratory environments[7-9]. The results were mutually supportive: In development, the enriched

animals showed more total sleep time, more REM sleep time, and longer REM sleep epochs. On daily swimming tests, Hodgson noted that enriched rats, who had enhanced REM sleep levels, also showed significantly greater swimming immobility than impoverished rats[6]. Importantly, in her work, enriched rats lost their advantage entirely when they were REM sleep deprived. It is concluded that environmental rearing condition (and the REM sleep enhancement which accompanies enrichment) is an important variable in elevating or depressing scores on the swimming immobility response[5,6].

The work of Mirmiran and his colleagues[10] is salient to all of the foregoing literature. They demonstrate that interference with the normal functioning of the REM sleep mechanism or interference with specific monoaminergic transmitter systems during early development, can produce long-lasting biochemical, brain morphological, and behavioral changes in later life. The evaluation of monoamine oxidase inhibitors and tricyclic antidepressant drugs is part of that context. Historically, our repeated measures protocol derives from a single-trial screening procedure pertinent to the evaluation of those drugs[11]. We have shown that REM sleep deprivation alone accounts for the drug test outcomes and that emotional defecation is a REM sensitive counterpart to the rat immobility changes described[2]. Recent work by van Luijtelaar and Coenen[12] confirms the tie between neuroleptic pharmaceuticals and the swimming test. Their results extend the idea of a common mechanism for the immobility and emotionality effects, whether the REM sleep deprivation is produced instrumentally or by drugs.

Hypothesis. The enrichment studies cited devolve directly from brain-developmental work. That work, in turn, was based on behaviorally selected, inbred, rat strains[13]. In this report we suggest parallel evidence regarding strain and vendor effects in the rat swimming model. It was expected that genetic (strain) differences might modify the response rate but not the occurrence of the immobility response. Our prior work has been based upon Sprague-Dawley rats obtained from a single, San Francisco Bay srea supplier (Simonsen Laboratories). Work done with a Sprague-Dawley stock in France[3] and a Wistar stock in The Netherlands[12] is in general agreement with our own. Nonetheless, since there is recurrent interest in the possibility of outcross contamination of commercial breeding stocks[14], we thought it worthwhile to make an intercontinental vendor comparison using the untreated (i.e., control) condition of the swimming test protocol[2]. Thus, in the present experiment, we directly compared rats born locally but derived from English (Bantin and Kingman Co.) Sprague-Dawley stock with the U.S. based Sprague-Dawley stock we have been using. These rats are albino. Also, though the literature has numerous examples of swimming test differences between domestic and wild rat stocks[15–17], strain difference effects have not been reported. We made strain comparisons by testing the F344 albino and the black-hooded Long Evans strains. We expected to find significant strain differences but not significant vendor differences on the immobility response.

Breeding histories. Altman and Katz[18] provide helpful summaries for some previous behavioral, biochemical, and physiological work on each of the rat stocks we have used. We cite them[18] for the following points regarding inbreeding histories. F344 (Fischer) is an inbred strain (i.e., all lineage is by sib matings). Inbreeding began in 1920 at Columbia University. F344 is presumed to be essentially homozygous (but see the caveat of Fitch[14]). Sprague-Dawley rats have been bred since 1924 but only as closed colony stock. Thus the homozygosity of their genome is not even theoretically known. Though not formally inbred, they may be relatively homozygous. They are highly uniform in appearance and are probably the most used rat research stock in the world. The Long Evans strain is inbred but has an unusual feature to its derivation. Long Evans rats derive from wild × albino-domestic crosses which were carried out in 1915. Brother to sister matings have been maintained since that



Mean swimming immobility times for the F344, Sprague-Dawley (SD) combined, and Long Evans (LE) rat stocks, over the seven days of testing. All sources of variance (Stocks, Days, Stocks X Days) were significant.

time. Thus, Long Evans represents the inbred strain with the closest 'wild rat ancestor'. Finally, it should be noted that PA is the only historically older rat strain (inbred since 1909). PA is albino and is described as 'healthy, vigorous, and vicious'. It derives from Wistar stock imported from Europe (location unspecified) to the University of Chicago in 1900.

Procedure. A reversed 12:12 light:dark cycle was maintained with food and water available at all but testing times. The animals had experienced at least 10 days of acclimation to the laboratory and 6 additional days of individual (5-min) handling. All were male rats between 45 and 47 days old as testing started. Sample sizes varied between 7 and 9 per group.

The test situation is relatively benign. A rat is dropped, tail first, from the top of an 18 cm × 40 cm (height) cylinder which contains a 15 cm high column of water maintained at 25 °C. The animals are able to touch bottom with their tail and hind feet but are also able to swim and dive with vigor. Soon the observer begins to see brief and later increasing episodes of the immobility response. This immobility is expected to increase both within a 5-min trial and across the seven days of repeated tests[2]. The total number of seconds of immobility in a 5-min trial is the dependent variable.

Results. As can be seen (fig.) all of the stocks confirmed the basic expectation in increasing scores for swimming immobility across days of testing. The Long Evans strain was markedly lower than all of the other groups for absolute response levels. They had no overlap with other groups on any trial day. A repeated measures ANOVA followed by t-tests (based on the error mean square for subjects) is reported. First, the ANOVA gave a significant outcome for the set of four rat stocks used [$F(3,28) = 4.78$, $p < 0.01$]. Then, we found that there is not a significant difference between the English derived and the U.S. derived Sprague-Dawley groups [$t(14) = 1.49$, $p > 0.05$]. Given this outcome, the two Sprague-Dawley groups have been combined for the figure. This, so that Sprague-Dawley can be seen most clearly in relation to the two inbred strains.

A significant difference was found with each comparison on the inbred strains. For the F344 vs Sprague-Dawley, $t(21) = 2.47$, $p < 0.03$ and for Long Evans vs Sprague-Dawley, $t(23) = 8.23$, $p < 0.001$. Long Evans an F344 were also significantly different [$t(14) = 9.03$, $p < 0.001$]. With regard to repeated measures, the Days component of the ANOVA (i.e., the expected replication) was significant [$F(6,168) = 28.96$, $p < 0.001$]. Finally, Stocks X Days also showed significance [$F(18,168) = 1.78$, $p < 0.03$]. This

indicates that the Long Evans rats have a different (flatter) trend line in addition to being more active overall.

Discussion. We note that all of the albino stocks tested show similar rates and levels of acquisition for the response. That is, the response differences between the F344 and the Sprague-Dawley rats seem only to be statistical, rather than qualitative differences. With the Long Evans strain, the interpretation is not as sure. These animals seem to be showing a slower acquisition pattern while also being less adaptive in terms of immobility. Since Long Evans is the only pigmented stock tested here, and since we have used only albinos in the past, we do not yet know whether the effect we are seeing is due to a pigmented retina (i.e., is it a visual acuity effect?) or whether it is a genotypic (i.e., a strain-typical) activity difference. Thus, we are content to make only two firm points with these data. First, given the similarity between the results from several laboratories in the past[3, 6, 12] with the results of the direct comparisons here, the swimming test can be expected to give a replicable outcome with a variety of rat stocks. Sprague-Dawley rats provide a standard outcome even when they are obtained through different vendor lines. For numerous independent variables then, the test should be effective regardless of locus of origin or the rat stock used.

Secondly, we suggest that this test protocol may provide good basis for continuing behavior genetic work using Norway rats in sleep research. Work with mice *(Mus musculus)* in that area of interest has produced much broadly valuable and elegant research, especially in the domain of psychopharmacology (see, e.g., ref. 19 and its references). In that regard, these data raise interesting questions with respect to albino vs pigmented phenotypes. Early mouse work had suggested that albino animals are more likely to show a passive or hesitant response pattern on a variety of sensory response and learning tasks[20, 21]. The immobility results are compatible with that expectation. Nonetheless, these rats may differ from mice or from other rat strains. As noted, PA is a very active rat strain even though it is albino. In that way it is more like the pigmented Long Evans strain. Thus, in future work, PA might show either the 'albino effect' or the 'strain difference effect' both of which are illustrated by the present data. In resolving that and other biobehavioral questions, we believe that the swimming model will be helpful in elucidating an adaptive, mechanistic, base for a variety of research questions. Traits which are REM sleep sensitive are especially implicated. This, because the REM sleep basis for both response acquisition and emotionality can now be studied simultaneously at the genetic, developmental, neurochemical, and behavioral levels with a reliable rat model.

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Bromocriptine and sulpiride competitively inhibit estrogen binding to its receptor in the adrenal gland

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Summary. Bromocriptine and sulpiride incubated simultaneously with [^{3}H]-estradiol in the cytosol from adrenal glands of adult male rats, yielded curves typical of competitive inhibition as analyzed by Lineweaver-Burk plots. The inhibition constant for both drugs was approximately 10^8 M^{-1}, only 10 times lower than the association constant for estradiol.
Key words. Estrogen receptors; adrenal; bromocriptine; sulpiride.

It is well known that estradiol plays a role in the regulation of the hypothalamic-hypophysial-adrenal axis. It has been reported that ovariectomy causes a decrease of plasma ACTH levels in rats[3]. Homogenates from ovariectomized rats showed a decreased in vitro corticosterone production when compared to homogenates from intact or ovariectomized animals injected with estradiol[4]. Since this effect was evident in homogenates from hypophysectomized-ovariectomized rats receiving both estradiol and ACTH, the estrogen action would appear to occur at least in part directly on the adrenal glands. In agreement with this, estrogen receptors (ER) have been identified[5] and characterized both in the cytosol and in extracts from nuclei of adrenal tissue by our group[6,7] and others[8,9]. Recently, we have studied prolactin regulation of ER in the adrenal gland[10]. This study demonstrated that bromocryptine (BC) and sulpiride (SP) had an effect on the endogenous levels of prolactin. In addition, the effects of these drugs upon adrenal weight and corticosterone secretion could not be ascribed to their action on prolactin levels[10]. Although it is possible that changes with ACTH serum levels occurred, these studies strongly suggest that BR and SP may have a direct effect on the adrenal gland through an interaction with the ER. To assess this hypothesis, we have evaluated