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Strain Specific Cholinergic Changes in Response to Stress: Analysis of a Time-Dependent Avoidance Variation[1]

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COOPER, D. O., D. E. SCHMIDT AND R. J. BARRETT. *Strain specific cholinergic changes in response to stress: Analysis of a time-dependent avoidance variation.* PHARMACOL. BIOCHEM BEHAV **19**(3) 457–462, 1983.—Investigators have established that the performance of an incompletely learned avoidance task is a U shaped function of the time since the original partial acquisition. Thus rats perform more poorly when retested at intermediate time intervals (1–8 hr) after training than they do when tested at longer post-acquisition intervals (24–48 hr). Studies have suggested that such time-dependent deficits are not related to changes in learning ability, but rather result from shock-induced motor suppression which interferes with active avoidance responding. Pharmacological studies utilizing drugs which effect cholinergic function have indicated that an inhibitory cholinergic system may be involved in mediating post-shock motor suppression. To obtain direct biochemical evidence for possible cholinergic mediation of post-shock motor suppression, measurements of high affinity choline uptake and acetylcholine turnover were made at varying time intervals following partial active avoidance training in F-344 rats. An increase in cholinergic function was found in the dorsal, but not the ventral hippocampus 30 min, 1 hr and 4 hr following acquisition training. These biochemical alterations were temporally correlated with deficits in active avoidance responding. We have reported that the immediate behavioral suppression observed in another rat strain (Sprague-Dawley, Zivic Miller Laboratories), which exhibits inferior active avoidance performance, is similarly correlated with cholinergic activation in the dorsal hippocampus [17]. These data support the hypothesis that the dorsal-hippocampal cholinergic system is involved in the mediation of stress-induced behavioral suppression. Furthermore, strain differences in the temporal response of this system following exposure to stress may partially underlie previously observed strain differences in active avoidance performance. Such strain differences in neurochemical function provide a useful model for further investigations of the mechanisms involved in the effects of stress on motor-dependent behavior.

Avoidance Stress Strain differences Acetylcholine

STUDIES in rats have repeatedly demonstrated that incompletely learned active avoidance tasks are subject to a time-dependent performance phenomenon known as the "Kamin Effect." Kamin [12] originally found that when rats are retested at intermediate intervals following partial active avoidance training they are inferior in performance to rats which are retested either immediately or 24 hr later. The maximal performance deficits in those rat strains which have been investigated occur between 1 and 6 hr after initial training [1–3, 19]. While various behavioral hypotheses have been advanced to explain this phenomenon (for review see [2]), recent studies indicate that shock-induced motor suppression may be responsible for time dependent deficits in avoidance performance.

Utilizing Y-maze brightness discrimination task, Barrett *et al.* [7] were able to demonstrate that F-344 rats which exhibited substantial performance deficits at intermediate time intervals after partial active avoidance training still remembered where to run, but had difficulty in initiating the motor activity required to make an avoidance response. Furthermore, they demonstrated that the occurrence of the motor suppression responsible for the observed performance deficits occurred as a function of the time since shock exposure, not the time since initial avoidance training. These data indicated, therefore, that the Kamin effect was not due to interference with associative processes, but rather suggested that shock-induced neurochemical alterations in the CNS produce a time-dependent behavioral suppression which results in subsequent performance deficits in incompletely learned tasks which require active responding. In conjunction with these studies, Barrett and co-workers [7,19] also described a strain of rat (Sprague-Dawley, Zivic Miller Lab-

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oratories; ZM) which was found to perform poorly in active avoidance tasks independent of the length of the retest interval. In fact ZM rats failed to demonstrate significant active avoidance acquisition in a Y-maze task even after many repeated daily training sessions. A variety of behavioral measures recorded in addition to the avoidance response itself indicated that these animals were unique in the rapidity with which they became immobile following the initial exposure to shock in a given training session. Thus, although this strain was able to readily learn where to run in the Y-maze to escape shock, the low probability of an active response virtually precluded their making contact with the avoidance contingency. In subsequent biochemical studies with ZM rats we have shown that there was a specific increase in dorsal hippocampal cholinergic function which was temporally correlated with the rapid onset of behavioral suppression in ZM rats [17]. This immediate change in cholinergic function was not observed in F-344 animals which alternatively, become more active immediately following shock.

More recently, in a series of studies by Anisman and coworkers, [2–6] it was demonstrated that in a variety of rodent strains the administration of shock produced neurochemical changes which resulted in deficits in the ability of these animals to initiate and/or maintain active motor responding. Moreover, these authors provided data which suggested that such temporal motor deficits resulted from stress-induced depletion of specific catecholaminergic pathways in brain. Prior exposure to unavoidable shock was also shown to result in a more rapid depletion of these pathways following a subsequent additional stress exposure, indicating that the neurochemical response of the system has become "sensitized" to repeated stress. In addition to the data on catecholaminergic function, these authors further suggested that a suppressive cholinergic influence may be involved in mediating stress induced changes in motor performance.

The present manuscript describes studies which demonstrate that the post-acquisition time-dependent reductions in active avoidance performance which can be observed in F-344 rats (i.e., the Kamin effect) are also temporally correlated with a specific increase in cholinergic function in the dorsal hippocampus.

METHOD

F-344 rats (Harlan Industries), 55–60 days of age were housed in groups of 4–5 per cage and were maintained on an 0700–1900 light cycle with food and water provided ad lib. One week of acclimation was allowed before experimentation.

Avoidance Procedure

A standard Sidman avoidance paradigm was used where rats were given training in a sound attenuated enclosure containing a 1 cubic foot Plexiglas cubicle with a floor composed of 16 stainless steel grids placed 1.5 cm apart. A scrambled shock (1.75 mA, 1 second duration) was delivered through the grid floor every three seconds if no responses were made. The shock could be avoided by pressing a bar located 4.9 cm above the floor. A bar press response delayed further shock delivery for 30 seconds. Avoidance responses (i.e., bar presses made during the 30 second interval) were calculated by subtraction of escape responses (i.e., bar presses made in response to shock) from total bar presses. All subjects were initially given 20 minutes of avoidance training and then removed from the apparatus and randomly assigned to retest or non-retest groups. The retest groups received 10 minutes of further testing either 0, 1/2, 1, 4 or 24 hr following acquisition. All retest animals were sacrificed immediately following the 10 minute retest interval. Animals in the non-retest groups were returned to their home cages and sacrificed at the appropriate post-acquisition intervals. A third group of cage controls, which were never exposed to shock, were sacrificed along with the retest and non-retest groups to obtain a biochemical baseline of cholinergic function.

High Affinity Choline Uptake

Following decapitation, hippocampi were rapidly removed and dissected over ice. Hippocampi were further dissected to separate the dorsal 1/3 from the ventral 2/3. Brains regions were then placed in 5 ml cold isotonic sucrose (0.32 M). Following homogenization (glass-teflon pestle), samples were centrifuged at 1000 × g for 10 minutes to remove cellular debris. Supernatants were then recentrifuged for 20 minutes at 22,000 × g. The resulting synaptosomal pellets were resuspended in 1 ml cold isotonic sucrose.

The rate of Na^+ dependent high affinity choline uptake (HACU) was measured by a modification [11] of the method of Kuhar *et al.* [13]. Briefly, 100 μl of the synaptosomal suspension was incubated with 0.9 ml sodium phosphate buffer or sodium free buffer (37°C) containing 10^{-6} M (0.4 μCi) ^{3}H-choline iodide (TRK-179, Amersham-Searle) in 1.5 ml Beckman microfuge tubes. Following 5 minute incubation, the reaction was terminated and the pellets re-isolated by three minute centrifugation in a Beckman microfuge. The supernatant was discarded by careful decantation and the pellets were gently surface washed twice with 1 ml cold isotonic saline. The bottom 2 cm of the tube was then cut and placed directly in 10 ml of ACS counting fluid (Amersham-Searle) for scintillation counting. Under these conditions, the rate of uptake was linear between 2 and 8 minutes. High affinity choline uptake (i.e., corrected for sodium free low affinity background) was calculated on the basis of cholineacetyltransferase activity. This method has been shown to be superior to HACU determined on the basis of total protein [11].

Acetylcholine Turnover

Relative acetylcholine (ACh) turnover was determined by measuring the rate of decline in ACh levels following intraventricular administration of hemicholinium-3 (HC-3) [16]. For determination of ACh turnover during shock administration, rats were given 20 μg HC-3, placed in the shock apparatus for 20 minutes and immediately sacrificed by head-focussed microwave irradiation [15]. Control rats were given HC-3, placed back in their home cages and similarly sacrificed 20 minutes later, in alternation with their shocked counterparts. For measurement of post-shock ACh turnover, at the appropriate time intervals after shock, both the shocked rats and their non-shock controls received HC-3 in alternation, were returned to their home cages and microwave sacrificed 20 minutes later. ACh levels were measured by pyrolysis gas chromatography as previously described [18].

Statistical Analysis

Analysis of variance, followed by either Newman-Keuls multiple comparison or Duncan's multiple range testing were used to compare the various control, retest and non-retest

groups for possible time dependent differences in avoidance behavior, Ach turnover or high affinity choline uptake.

RESULTS

Figure 1a and 1b represent the mean number of bar presses made and mean number of shocks received, respectively, during the two 10 min acquisition periods, as well as during the 10 min retest session at the indicated training-retest time intervals.

There were no significant differences among the groups during either the first or second 10 min acquisition periods on either the shock or bar press measure. Thus, the acquisition means presented in Fig. 1a and 1b represent the means from the pooled values from all subjects. Using t-tests for correlated means to compare performance between the first and second 10 min periods it was found that the subjects made more bar presses, $t(39)$ 6.10, $p<0.01$, and received significantly fewer shocks, $t(39)=11.06$, $p<0.01$, during the second 10 min of acquisition than during the first 10 min, indicating that the subjects were learning the avoidance contingency.

One way analysis of variance computed on the retest data showed that both the number of shocks received, $F(4,35)=6.57$, $p<0.01$, and the number of avoidance responses made, $F(4,35)=7.4$, $p<0.01$, varied as a function of the training-retest interval. As can be seen in the figures, the number of responses was greatest at the immediate retest interval and fewest at the 1 hr interval, while the opposite was true for the shock data.

Separate repeated measures analysis of variance were computed comparing the first and second ten min acquisition periods with the 10 min retest session on both shock and bar press measures. Analysis of the bar press data indicated a significant retest interval × test session (acquisition or retest) interaction, $F(4,35)=8.4$, $p<0.01$, which was due primarily to the greater number of bar presses made by the immediate retest group compared to their acquisition data. The same analysis, comparing the second 10 min of acquisition with the retest data, also revealed a significant retest interval × test session interaction, $F(4,35)=36$, $p<0.05$. Subsequent tests on the simple main effects showed that the 1, 4 and 24 hr groups made significantly ($p<0.05$) fewer avoidance bar presses during the retest session than during the second 10 min of acquisition. The results from the same analysis on the shock data indicated that the subjects in the immediate retest groups received fewer shocks during retest, $F(4,69)=90$, $p<0.05$, than during the first 10 min of acquisition. However, the rats in the 1 hr, $F(4,69)=11.25$, $p<0.01$, and 4 hr, $F(4,69)=7.3$, $p<0.05$, groups received a significantly greater number of shocks during retest than they had experienced during the first 10 min of acquisition. Tests comparing the retest data with the second 10 min of acquisition revealed that rats tested ½ hr, $F(4,66)=7.82$, $p<0.05$, 1 hr, $F(4,66)$ 21.6, $p<0.01$, and 4 hr, $F(4,66)=18.3$, $p<0.01$, following acquisition received significantly more shocks during retest, while the number of shocks received by the immediate and 24 hr retest group were not different from the number received during the second 10 min acquisition session.

The time dependent alterations in dorsal hippocampal HACU are presented in Fig. 2. Three × five analysis of variance reveals significant group, $F(2,105)=7.701$, $p<0.01$, and time, $F(2,105)=5.780$, $p<0.01$, effects. Subsequent tests on simple main effects showed a significant variation of the retest group across time, $F(4,102)=4.80$, $p<0.01$, while

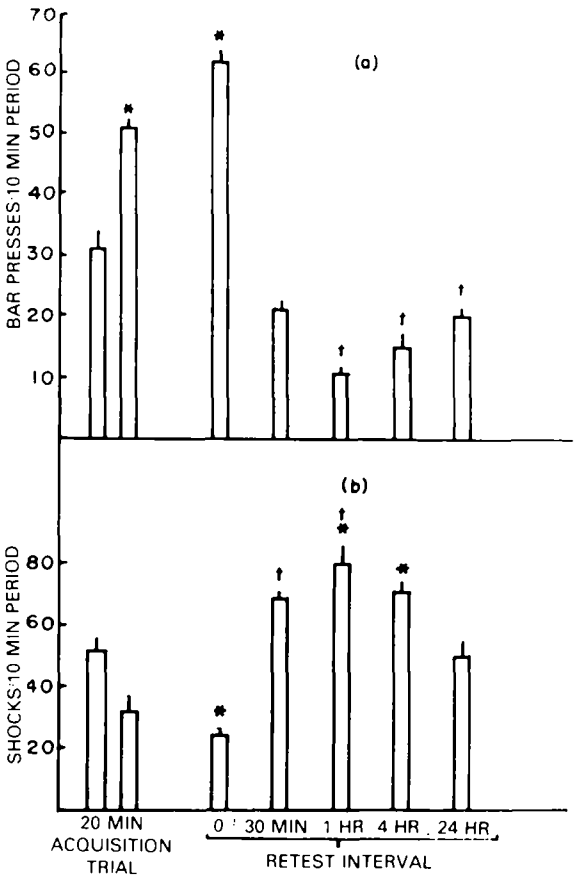

FIG. 1. Mean number of bar presses±SEM (a) made and shocks+SEM (b) taken during a 20 minute Sidman avoidance acquisition period and during subsequent retesting. A total of 40 rats were given initial training and sub-groups of 8 rats were then retested at the indicated times following training. There was no difference in the 20 minutes acquisition behavior of the sub-groups and the acquisition curve represents the pooled data from all rats. *$p<0.05$ vs. the first 10 minutes of acquisition learning. †$p<0.05$ vs. the second 10 minutes of acquisition learning.

neither the control nor the non-retest groups varied significantly as a function of time. Newman-Keuls multiple comparison testing further revealed that at both the 1 hr and 4 hr time point, choline uptake values for the re-test group were significantly different ($p<0.05$) from either control or non-retest values. Similar analysis also revealed that the rate of choline uptake for the retest group at the 4 hr period was significantly different from uptake values for the retest groups at all other time intervals ($p<0.05$).

Figure 3 represents the values for the hippocampal ACh turnover. Analysis of variance revealed a significant time, $F(3,56)=7.61$, $p<0.01$, and group, $F(1,56)=5.34$, $p<0.05$, effect and a significant time × group interaction, $F(3,56)=6.73$, $p<0.05$. Further analysis using the Duncan multiple range testing revealed that at both the 60–80 min and the 120–140 min post shocks interval hippocampal ACh turnover was significantly elevated ($p<0.05$).

DISCUSSION

The data presented here provide direct biochemical evi-

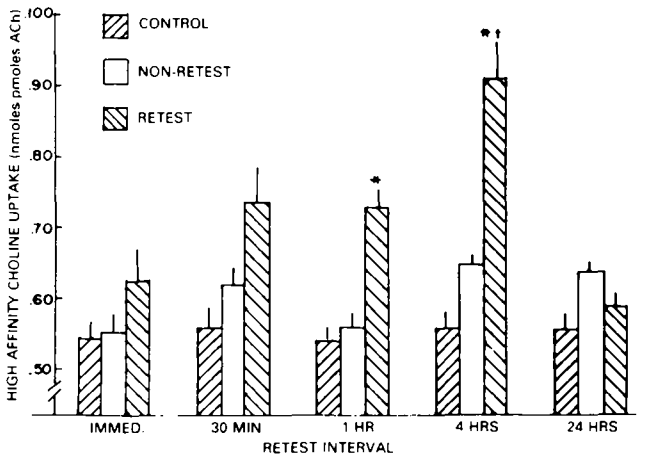

FIG. 2. The change in Na^+ dependent high affinity choline uptake in dorsal hippocampus following Sidman avoidance training. Eighty rats were given 20 minutes of Sidman avoidance training and divided into 5 retest and 5 non-retest groups of 8 rats each. At the indicated intervals after training the retest groups received an additional 10 minutes Sidman training and were then sacrificed in alternation with the non-retest group and a group of 8 home cage controls which received no Sidman training. Following sacrifice, dorsal hippocampus was removed and HACU measured as indicated. Choline uptake is expressed as pmol choline/nm ACh synthesized ± SEM. *$p<0.05$ vs. control and non-retest groups. †$p<0.05$ vs. 0, 30 min, 1 hr and 24 hr retest groups.

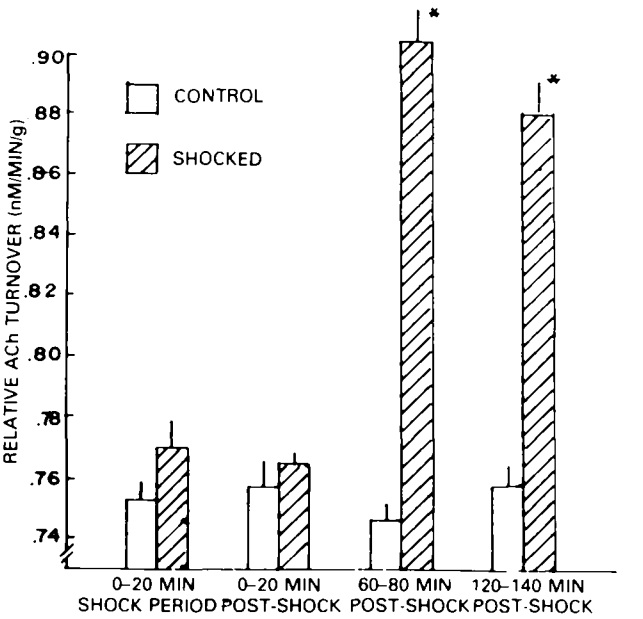

FIG. 3. Relative ACh turnover in dorsal hippocampus following Sidman avoidance training. Control rats received only 20 μg HC-3 and were returned to their home cage for 20 minutes prior to sacrifice. The 0–20 minutes shock period rats received 20 μg HC-3 and then received 20 minutes Sidman avoidance training prior to sacrifice in alternation with control group rats. Post-shock rats received 20 μg HC-3 at 0, 60 or 120 minutes after Sidman training, were returned to their home cage for 20 minutes prior to sacrifice in alternation with control group rats. Each group contained a minimum of 8 rats. *$p<0.05$.

dence for a temporal relationship between increases in dorsal-hippocampal cholinergic function and the behavioral suppression which occurs at specific time periods following shock exposure in F-344 rats. These increases in HACU and ACh turnover in the dorsal hippocampus are correlated with deficits in avoidance performance at 1 and 4 hr following initial avoidance training. The behavioral data demonstrate that when the acquisition of active avoidance by F-344 rats is incomplete (i.e., further exposure to the correct contingency is required), the time dependent suppression of motor activity which occurs subsequent to shock stress is incompatible with such successful exposure and deficits in acquisition become apparent. These deficits are quite dramatic when it is considered that, despite 20 min of prior exposure to the avoidance contingency, groups of F-344 rats retested at intermediate time intervals display less ability to avoid than do completely naive rats (i.e., first 10 min of acquisition; Fig. 1).

We have previously reported that the marked motor suppression which occurs in another strain of rats (Zivic Miller, Sprague-Dawley) as an immediate response to shock is also correlated with an increase in dorsal hippocampal cholinergic function [17]. The rapid behavioral suppression seen in Z-M rats is also incompatible with the motor activity which is necessary to discover avoidance contingencies and therefore results in inferior avoidance acquisition by Z-M rats. The correlation of stress induced motor response suppression and alterations in dorsal hippocampal cholinergic function in two behaviorally distinct rat strains therefore provides evidence that activation of an inhibitory cholinergic system in the dorsal hippocampus participates in production of motor suppression and suggests that temporal changes in this system may be involved in regulating stress-induced, time dependent alterations in active avoidance behavior (i.e, such as Kamin effect).

Cholinergic motor response inhibition, functioning to oppose catecholaminergically mediated motor response activation as part of a balanced system to regulate response rates was first suggested by Carlton in 1963 [9,10]. This model, and other subsequent hypothetical models (see recent review [2]) suggest that shock exposure during active avoidance training elicits catecholamine release which produces behavioral arousal and increased motor activity. It is further hypothesized that this catecholamine release results in a homeostatic rebound of inhibitory cholinergic function resulting in subsequent attenuation of the level of shock induced motor activity. Such a simple model, in which cholinergic inhibition functions only as a direct inhibitory feedback loop opposed to catecholaminergic activation does not, however, provide an adequate explanation for the neurochemical events which we have observed in F-344 and Z-M rats. F-344 rats which were not re-stressed (i.e., not retested, but sacrificed at appropriate post-acquisition intervals) but which were similarly shocked during acquisitions trials, did not show a significant increase in HACU as was observed in their retested counterparts. If suppressive cholinergic activation were occurring as a simple time-dependent inhibitory rebound to catecholamine release resulting from the initial stress episode, an increase in HACU should also have occurred in the non-retest group. Rather, the system appears to operate such that in the F-344 rats, the initial shock exposure "sensitized" the regulation of dorsal hippocampal cholinergic neurons so that they were more

readily activated upon re-exposure to subsequent stressful stimuli. Such sensitization may also be responsible for the statistically insignificant but very consistent increases in HACU values which were observed in the non-retest group vs. the control rats. A similar sensitization of catecholaminergic systems to more rapid depletion by repeated stressful events has also previously been demonstrated (for review, see [2]).

The motor behavior of Z-M rats during initial shock exposure further suggests that a simple inhibitory feedback model for cholinergic motor suppression is not completely satisfactory. When Z-M rats are exposed to shock stress they do not experience initial motor activation, but rather, immediately become suppressed. It is difficult to understand how such an immediate suppression, which was also correlated with an immediate increase in dorsal hippocampal cholinergic function [17], could occur as a secondary event to catecholaminergically mediated arousal when such arousal appears to be absent. It is more parsimonious to suggest that in these rats cholinergic inhibition occurs as a primary reaction to stress.

These data, therefore, while not inconsistent with a catecholaminergic-cholinergic balance as a factor in regulating behavioral arousal, nor with stress-mediated catecholamine release, do alternatively suggest that the cholinergic suppressive portion of such a balanced system is independently regulated and may have a primary, rather than just a feedback role, in governing the level of behavioral response to stressful stimuli.

The ACh turnover data also provide indirect evidence which suggests the time-dependent sensitization of the hippocampal cholinergic system seen following shock stress in F-344 rats is not specific only to shock but will occur in response to other forms of stress as well. In making the ACh turnover measurements which shows a significant increase in ACh turnover at the indicated time intervals following acquisition trials, the data presented in Fig. 3 was determined in non-retested rats. This is in contrast to HACU data, in which a significant increase in choline uptake was observed only in rats which were retested (i.e., re-stressed). We believe that the answer to this apparent paradox lies in the method required for estimating ACh turnover. To make such determinations, rats were taken at appropriate intervals following initial shock exposure and, while conscious, were given intraventricular injections of HC-3. This procedure requires extensive handling and close restraint for a period of several minutes. That these rats show increased ACh turnover compared to rats which were similarly handled and injected with HC-3, but which were not subjected to prior shock exposure suggests to us that the increase in ACh turnover, similar to that seen in HACU in re-shocked rats, represents a "sensitized" cholinergic response to handling stress and is evident only at the appropriate times following the initial shock exposure. Thus, the stress of the HC-3 injection procedure produced time-dependent cholinergic changes similar to those seen in response to the stress of additional avoidance training.

On the behavioral level, the demonstration of a time-dependent performance deficit in a Sidman avoidance paradigm provides information on the probable importance of shock-induced behavioral suppression in governing responses to stressful stimuli. Previous studies have primarily utilized shuttle box or Y-maze avoidance paradigms which require substantial locomotor activity for successful avoidance. That significant performance deficits are also readily apparent in a paradigm which requires little locomotor behavior for successful avoidance suggests that a wide range of response repetories may be sensitive to shock-induced suppression.

In summary, we have presented biochemical and behavioral evidence which support the hypothesis that the temporally distinct behavioral suppression which can be observed in various rodents strains following stress is mediated, at least in part, via activation of a suppressive dorsal-hippocampal cholinergic system. Moreover, the data suggest that such cholinergic activation does not occur simply as a direct homeostatic negative feedback response to catecholaminergic behavioral excitation, but rather, is a complex and independently regulated response control pathway which shows dramatic variation between rat strains.

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