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Effects of Caffeine and L-PIA on Rats With Selective Damage of the Hippocampal System[1]

RONALD N. SHULL[2] AND FRANK A. HOLLOWAY

Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center Oklahoma City, OK 73190

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SHULL, R. N. AND F. A. HOLLOWAY. *Effects of caffeine and L-PIA on rats with selective damage of the hippocampal system.* PHARMACOL BIOCHEM BEHAV 22(3) 449–459, 1985.—Rats with electrolytic lesions of either the medial septum or hippocampus or with colchicine-induced lesions of the dentate granule cells produced significantly higher amounts of lever pressing as compared to controls on both VI50 sec and VT50 sec reinforcement schedules. Caffeine, at doses of 3.2, 10, and 32 mg/kg, produced a dose-related decrease in operant responding for all lesioned animals while having little effect on the responding of controls. L-PIA (an adenosine analog), at doses of 0.01, 0.05, and 0.10 mg/kg, produced similar but more variable effects in some of the groups tested and did not alter the rate-reducing effects of caffeine (32 mg/kg) when given concurrently (0.05 mg/kg). Caffeine administration also appeared to coincide with a long-term decrease in response rates that continued after cessation of its administration. Explanations are advanced as to caffeine's possible mode of action in this experiment. The postulate that colchicine-induced damage of dentate granule cells might be a viable animal model of some forms of hyperactivity also is advanced. This specific lesioning procedure, by itself, produced animals which displayed significantly higher than normal levels of general activity which were also sensitive to caffeine's rate-decreasing effects.

Hippocampus Medial septum Dentate gyrus Granule cells Caffeine L-PIA Operant behavior General activity Hyperactivity Colchicine

DRUGS that stimulate the CNS to higher levels of "activity" have been used in various situations to facilitate recovery from brain damage, with two of the most common classes of substances being the amphetamine compounds and the anticholinesterases [7]. Another drug which has been used with some success to at least compensate for some of the symptoms of brain damage is caffeine. Luria [16] cites an example where caffeine administration was used to correct to some extent the visual perception difficulties of a patient with occipito-parietal injury. Caffeine itself is a somewhat powerful CNS stimulant which appears to affect all portions of the cerebral cortex and tends to produce a more rapid and clearer flow of thought while reducing fatigue and drowsiness in humans [10]. It appears to produce its various behavioral effects by several means, including preventing cyclic AMP destruction via brain cyclic AMP phosphodiesterase, releasing central catecholamines which then stimulate the receptor enzyme adenylate cyclase (increasing cyclic AMP), competitively antagonizing brain adenosine receptors, and increasing the availability of intracellular calcium in some tissues [2].

Of these various processes, caffeine blocks adenosine receptor activity in concentrations similar to those which produce behavioral stimulation and, because the general neurophysiological actions of adenosine are inhibitory, it is conceivable that caffeine produces its major excitatory behavioral effects by blocking adenosine receptors [18]. In this regard, the recent discovery of high densities of adenosine receptors in both the limbic system and basal ganglia of the rat, especially in such areas as the molecular and polymorphic layers of Ammon's horn and the dentate gyrus, is especially interesting [11].

Damage to various components of the hippocampal system has been directly correlated with various disturbances in behavior. One of the most prominent behavioral effects seen has been characterized as either a lack of behavioral inhibition or an increase in behavioral perseveration. Kimble [13] proposed that the mammalian hippocampus constituted a portion of the neural machinery necessary for the generation of a brain process that was functionally equivalent to Pavlovian internal inhibition. Damage to this area, then, would impair the normal "behavioral braking" provided by this internal inhibitory mechanism and the animal would be less able to inhibit or alter its responses or attention to initially prepotent environmental stimuli and thus display less flexible and, subsequently, less adaptive behavior [13]. A recent

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[2]Requests for reprints should be addressed to Ronald N. Shull, Dept. of Psychiatry and Behavioral Sciences, Univ. Of Oklahoma Health Sciences Center, OMH Research Building, 306R, P.O. Box 26901, Oklahoma City, OK 73190.

example of this lack of inhibition was provided by Devenport and Holloway [3] who demonstrated that unlike control animals, rats suffering from massive hippocampal lesions do not reduce their lever pressing for food pellets even when pellet delivery is no longer contingent upon operant responding of any kind.

In a similar vein, Douglas has suggested [5] that the hippocampus has two different but related modes of functioning. One consists of the nonspecific inhibition of general emotional reactivity that requires only that the pyramidal cells be functional and driven by some synchronous input such as the theta-pacing system originating in the medial septum. The other consists of specific inhibition of an emotional reaction to a particular stimulus which requires that the pyramidal cells receive relevant stimulus information from the neocortex via the temporammonic tract and its major target, the dentate gyrus. In this regard, it has been observed that specific lesions of the septum and dentate gyrus produce changes in behavior similar to those seen with larger, less specific hippocampal lesions, thus implicating these other neural areas of the hippocampal system in the control of response "braking" in the aroused animal [19].

The above information led us to wonder if various substances which reportedly affect adenosine receptors would alter behavior that was at least partially modulated by the hippocampal system. More specifically, it seemed quite possible that these substances could perhaps alter or even reverse the behavioral effects seen with damage to this system. In order to test this idea, we chose an operant paradigm to observe the effects of both caffeine and the adenosine analog L-PIA (L-N6-phenyliso-propyladenosine) on "abnormal" behavior generated by rats with selective damage of various specific areas of the hippocampal system. Within this paradigm, we utilized both a variable interval and a variable time schedule which also allowed us to view the effects of the various surgical and drug procedures on the development of "superstitious" behavior (i.e., no reduction in operant rate with schedule changes from VI to VT) as described by Devenport and Holloway [3].

METHOD

Subjects

Twelve experimentally naive adult male Sprague-Dawley rats (Sasco, Inc.), weighing between 313 and 367 grams at time of surgery, were used. After surgical recovery and prior to training, all animals were reduced to approximately 80% of their free-feeding weight and maintained at that point by supplemental feeding which allowed for continued growth. All animals were housed individually in standard rack cages with ad lib water in a temperature controlled animal room maintained on a 12:12 light/dark cycle with light onset at 0800 CST.

Surgical Procedures

Each rat was randomly assigned to one of four experimental groups. All surgeries were performed under sodium pentobarbital anesthesia (42 mg/kg, IP) and utilized a stereotaxic instrument. Three rats (MS group) received electrolytic lesions of the medial septum (1.5 mA for 10 sec each site) utilizing a No. 00 stainless steel insect pin (insulated except for 0.5 mm at the tip) which was positioned at two sites. With bregma 1 mm above lambda, the coordinates of the rostral site were 0 mm posterior to bregma, 0 mm lateral to midline, and 5.5 mm below the cortical surface; while the caudal site coordinates were 0.5 mm posterior to bregma, 0 mm lateral to midline, and 4.6 mm below the cortical surface.

The next three rats (HC/E group) received bilateral electrolytic lesions of the hippocampus in toto (1.5 mA for 40 sec at each rostral site, 50 sec at each caudal site) utilizing the same electrode described above. Coordinates for the rostral site were 2.6 mm posterior to bregma, ±1.6 and 2.6 mm lateral to midline, and 3.6 and 3.2 mm below the horizontal surface of the skull, respectively. Coordinates for the caudal sites were 4.7 mm posterior to bregma, ±4.6 mm lateral to midline, and 4.0 and 7.2 mm below horizontal skull surface.

The next lesion group consisted of three rats (HC/C group) which each received bilateral intracerebral injections of colchicine (3.5 μg in 0.7 μl of distilled water at the rate of 0.1 μl/5 sec) at four separate sites within the dentate gyrus. With bregma 1 mm above lambda, the coordinates of the two rostral sites were 3.5 mm posterior to bregma, ±1.8 mm lateral to midline, and 3.3 mm below the cortical surface, while the caudal site coordinates were 4.5 mm posterior to bregma, ±4.5 mm lateral to midline, and 5.5 mm below the cortical surface. All animals were allowed a recovery period of at least two weeks. The remaining three rats were not operated upon and became the control (CON) group.

Apparatus

Behavioral measurements were made in four Lafayette (modular type) operant chambers, each equipped with a lever located above and to the right of a food cup which accepted 45 mg Bio-Serve pellets delivered by Lafayette dispensers. A photodetection system was installed in opposing chamber walls parallel to, but 25 cm away from, the chamber wall containing the lever and food cup. The beam from the light source to the photocell was 3 cm above the chamber floor and 5 cm from the back wall. Interruption of this photobeam was intended to provide numerical data on that portion of an animal's activity within the chamber which was not directed toward the lever or food cup and subsequent testing has shown that it is very difficult for a rat engaged in lever pressing to simultaneously activate this photodetection system. Chamber lighting was provided by a dim illumination through the translucent ceiling of each chamber. Each chamber was enclosed in a sound and light-attenuating box of local manufacture and programming and recording were handled by LVE/BRS component electronics.

Procedure

After the animals were reduced to 80% of their free-feeding weight, they were trained to lever press in the chambers described above. After training was completed, each subject was tested on alternate days (every 48 hours) at the same time each day, with one member of each experimental group being tested at the same time, and with the number of lever presses and photocell interruptions per session being recorded for each subject. The MS, HC/E, and CON groups were first tested on extended sets of both variable interval (VI) 50 sec (contingent) and variable time (VT) 50 sec (noncontingent) schedules in which 50 food pellets were delivered between intervals varying from 5 to 200 seconds. Each session lasted approximately 41.7 minutes and there were 5 sessions per schedule. Since the HC/C group was not included in this extended testing, however, this data was not included in the present experimental analysis.

Following completion of this, twelve separate drug treatment conditions, each consisting of two alternating sessions

of each of these schedules (termed a treatment cycle) utilizing an A(VI)-B(VT)-A-B design, were tested (same schedule and session parameters as above). The first treatment cycle consisted of a nondrug baseline (no injections given), which was then followed by three separate drug treatment cycles utilizing IP injections of caffeine dissolved in normal saline, given 20 minutes before testing, at dose levels, in order of delivery, of 3.2, 10, and 32 mg/kg. Each dose was utilized for all twelve subjects during a single ABAB testing cycle such that each cycle became a treatment cell for each animal in an extended progressive dose-effect curve. After a six day rest period (no testing or injections), a fifth treatment cycle, consisting of another nondrug baseline, commenced, followed by three more drug treatment cycles utilizing IP injections of L-PIA in normal saline 20 minutes before testing, at 0.01, 0.05, and 0.10 mg/kg dose levels, using the same sequence described above for caffeine. After another six day rest period, a third nondrug baseline treatment cycle was initiated, followed by a mixed drug treatment cycle in which 0.05 mg/kg of L-PIA was injected IP 30 minutes before testing, followed by a 32 mg/kg IP injection of caffeine 10 minutes later. A second caffeine-alone treatment cycle (32 mg/kg) followed this, after which a final nondrug treatment cycle (the MBL cycle), following another six day rest period, commenced, utilizing a mixed and extended schedule arrangement (B-A-A-B-B-A). It has previously been demonstrated [17] that after a 32 mg/kg IP injection of caffeine, blood levels and behavioral effects do not change appreciably during the post-injection interval used in this experiment (20–60 min).

Histology

After completion of the behavioral testing, all lesioned animals were perfused via cardiac puncture while under a lethal dose of sodium pentobarbital. The brains were subsequently removed and fixed in a buffered 10% formalin/sugar solution. Serial sections of 80 μ thickness were cut on a freezing microtome and every other section through a respective lesion was stained with cresyl violet after having been slide mounted. Lesion reconstruction was carried out using a projection microscope.

Statistical Analysis

On every behavioral session both lever-pressing and activity were monitored. It should be noted that every data point for both the latter measures was replicated across all drug/dose conditions and both schedule conditions (VI and VT) for all subjects in each group. Since the replicates produced essentially identical results, a mean for each set of replicate samples was obtained and these means were then used in the subsequent statistical analyses. Separate three-way analyses of variance (group, schedule, drug) were performed on the response and activity measures for the following sets of drug conditions: (a) Baseline-1 and the three doses of caffeine; (b) Baseline-2 and the three doses of L-PIA; (c) Baseline-3, 32 mg/kg caffeine + L-PIA, and 32 mg/kg caffeine alone; and (d) Baseline-1, Baseline-2, Baseline-3, and Baseline-4. The F-ratios for simple effects (group, drug, schedule) were determined using the appropriate pooled error term recommended by Winer [21]. Duncan's Multiple Range Test was used to compare separate cell means within each factor.

RESULTS

Histology

Figure 1 shows the major extent of lesioning in all three animals of the MS group. All three of these animals received substantial damage to the medial septum with most of the incidental damage surrounding this area and extending caudally beyond the septum within the same plane. None of them displayed any signs of post-surgical hyperaggression and appeared to be less reactive to handling than controls.

Figure 2 shows the major extent of lesioning in all three animals of the HC/E group. All three of these animals received at least some damage to both dorsal and ventral hippocampus although the focus of damage varied among the subjects, especially in the more caudal regions. In all cases, these electrolytic lesions left at least some of the hippocampus intact, although no definite functional differences could be assigned to any particular pattern of lesioning. They displayed greater than normal post-surgical weight loss and were much more reactive to handling than controls.

Figure 3 shows a comparison between the dorsal hippocampus of a rat from the MS group (A) and that same area in a rat from the HC/C group (B). Note that the major observable difference between the two is a complete atrophy of the dentate gyrus, most specifically the granule cells, of the HC/C group member. One also can see some shrinkage of the alveus and loss of both size and demarcation in the area between Ammon's horn and the dentate gyrus which might indicate indirect damage due to the loss of dentate cellular activity. No other form of neural damage could be observed in any of the HC/C group animals and this specific type of lesion matches very well with that described by Goldschmidt and Steward [9], who also used colchicine to preferentially destroy the granule cells of the dentate gyrus. Their reaction to handling appeared comparable to controls and no hyperaggression was seen. However, they and the HC/E group displayed a great deal of food spillage which was not seen in either the MS group or the controls.

Caffeine Dose-Effect Analysis—Operant Responding

The top two sections of Fig. 4 show the dose-related effects of caffeine on response rate under the VI and VT schedules. Caffeine produced an overall dose-related decrease in responding, $F(3,24)=14.68$, $p<0.01$. A significant dose-related decrease was noted for all lesion groups ($df=3/24$; $F(MS)=4.35$, $p<0.05$; $F(HC/E)=6.31$, $p<0.01$; $F(HC/C)=7.33$, $p<0.01$) but not for the control group and was apparent with each schedule condition (all p's<0.01). Significant differences among groups were found for both schedules at the baseline and 3.2 mg/kg caffeine dose (all F's$(3,56)>5.00$, $p<0.01$) but only for the VI schedule at the 10 mg/kg dose, $F(3,56)=5.04$, $p<0.01$. No differences among lesion groups were noted, but at the Baseline 1, and the 3.2 mg/kg and 10 mg/kg dose conditions all lesion groups had significantly higher response rates than the control group under both the VI and VT schedules (all p's<0.05). Overall, the VT schedule of food delivery resulted in fewer responses than did the VI schedule ($F(1,8)=710.84$, $p<0.01$); a similar effect was apparent in all groups (all p's<0.01).

In summary, all lesion groups had significantly higher levels of responding than did control animals under both contingent (VI) and non-contingent (VT) schedules of food delivery, but the highest dose of caffeine reduced responding to a level not significantly different from controls.

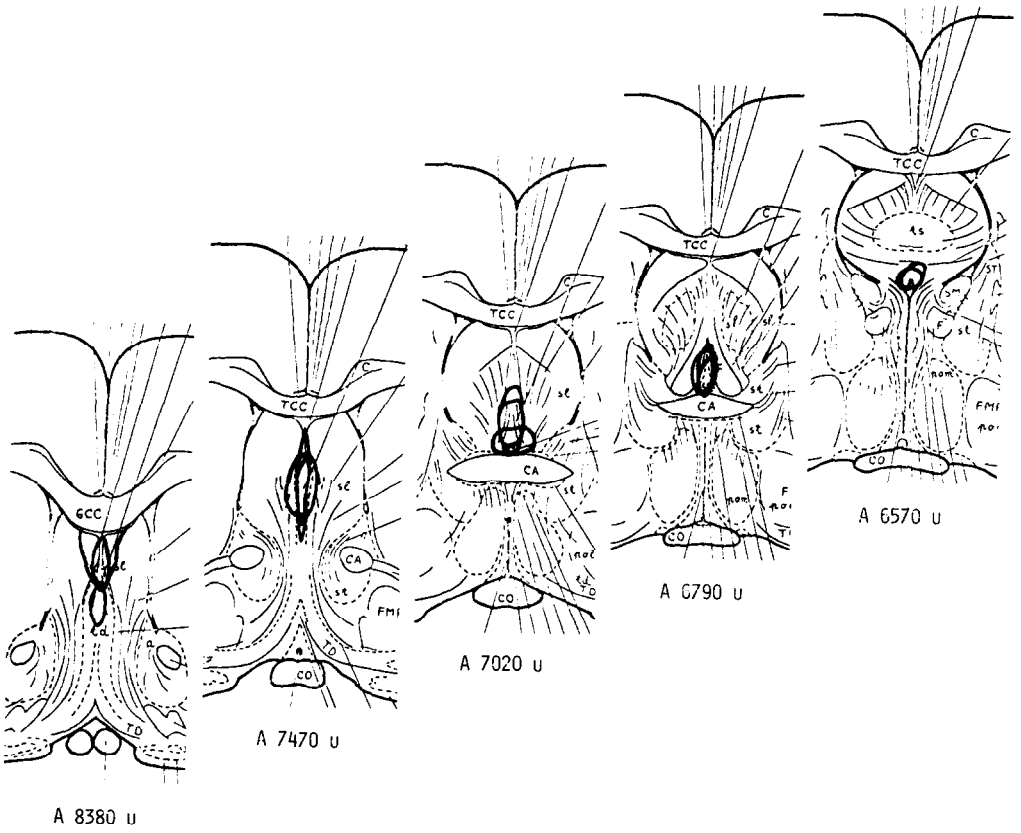

FIG. 1. Schematic representation of selected frontal sections showing the placement of lesions in the MS group (N=3). Schemas were taken from Konig and Klippel [14]; refer to that source for identification of structures. The legends indicate the distance of the presented planes from zero plane.

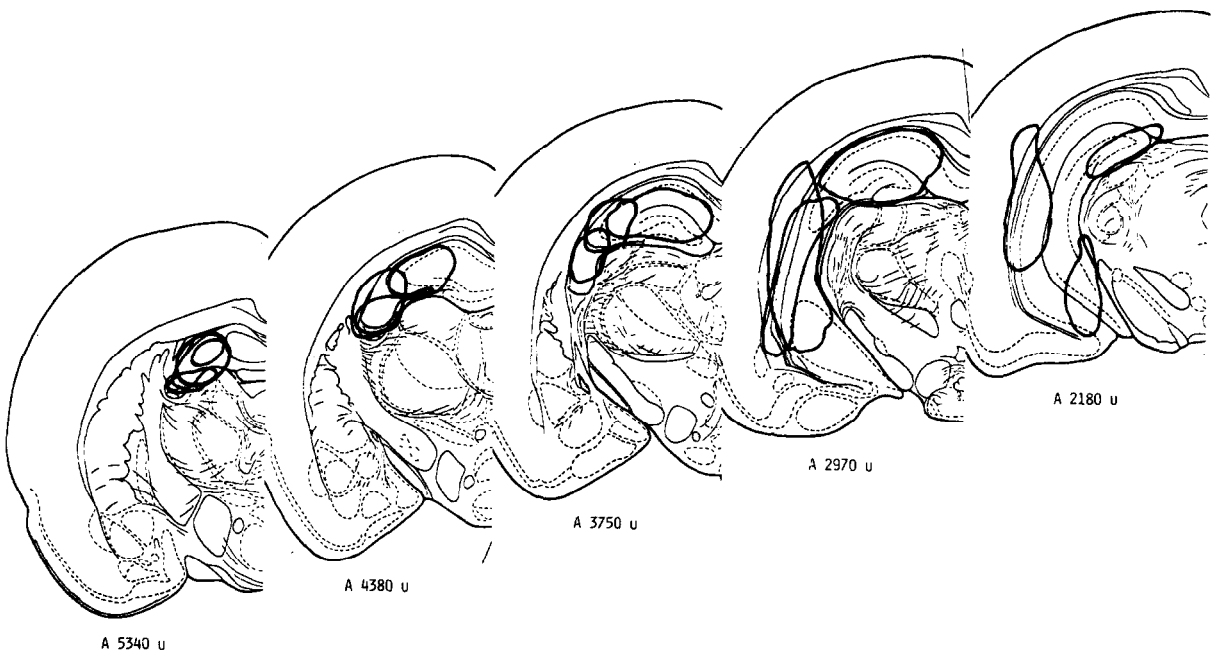

FIG. 2. Schematic representation of selected frontal sections showing the placement of lesions in the HC/E group (N=3).

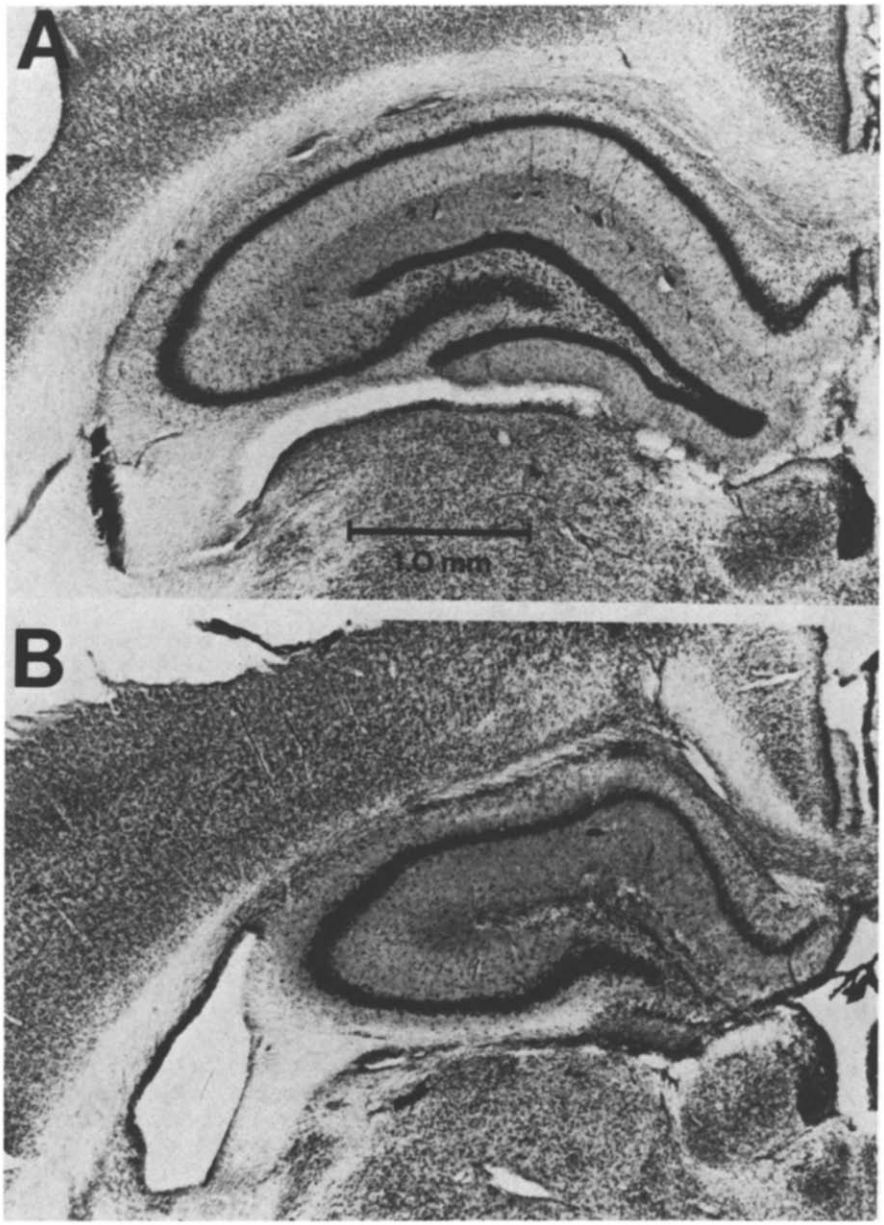

FIG. 3. Photomicrograph of a cresyl violet-stained frontal section at the level of the dorsal hippocampus showing Ammon's horn and the dentate gyrus of a representative sample of both an MS (A) and HC/C group member.

Caffeine Dose-Effect Analysis—Activity

The two bottom sections of Fig. 4 show the non-operant activity (photocell) data obtained during Baseline-1 and after various doses of caffeine (same sessions previously described). The greatest ANOVA main effect was found for groups, $F(3,8)=41.82$, $p<0.01$. The HC/C (colchicine) group produced significantly higher activity scores than all other groups at all dose levels of caffeine and under both schedule conditions (all p's<0.01 except for the 32 mg/kg dose and VT schedules where p's<0.05). There was a significant triple interaction main effect (groups/drug dose/schedule): $F(9,24)=4.82$, $p<0.01$). The latter interaction reflects the fact that the HC/C group's activity scores were significantly reduced by the 32 mg/kg caffeine dose but only under the VT schedule conditions, $F(3,56)=3.22$, $p<0.05$. No significant differences in activity scores between the two schedule conditions were found for any group at any level of analysis.

Thus, in distinction to the operant response measure, the operant chamber activity measure indicated that only the HC/C lesion group differed significantly from controls (and the other lesion groups too) and that this group's elevated activity scores were reduced by the highest dose of caffeine only under conditions of non-contingent food delivery.

L-PIA Dose-Effect Analysis—Operant Responding

The second phase of this experiment examined the

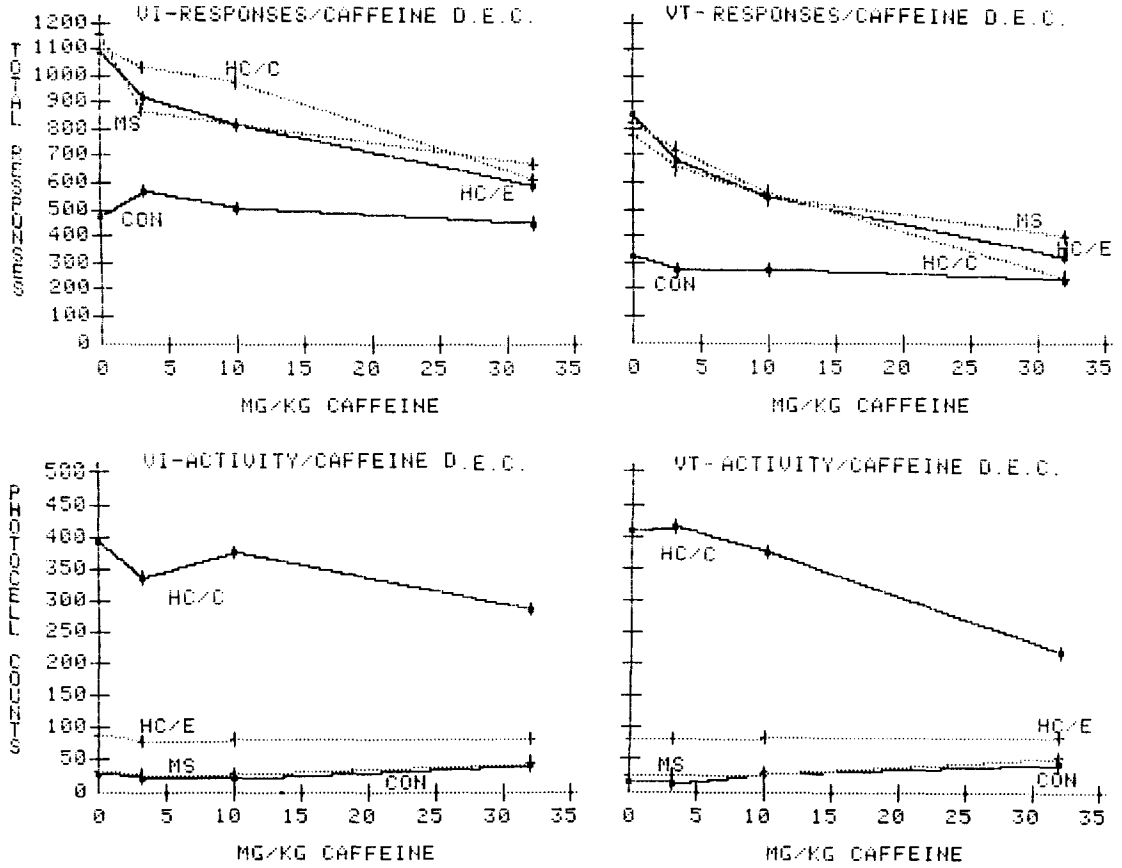

FIG. 4. Mean operant responses (lever presses) (top) and non-operant activity levels (photocell counts) (bottom) per session during both the contingent VI 50 sec (left) and non-contingent VT 50 sec (right) schedules after the IP administration of caffeine. Values represent replicate means with N=3 at each point (0=first baseline). See Results section for group designation.

dose-effects of L-PIA on operant responding and concomitant non-operant activity. The mean operant responses for all groups and schedules under Baseline-2 and the several doses of L-PIA are presented in the two top sections of Fig. 5. A weak overall dose-related effect of L-PIA was found, $F(3,24)=2.63$, $p<0.10$. However, only the HC/E group displayed an overall dose-related decline in responding, $F(3,24)=2.96$, $p<0.10$. The latter L-PIA dose-effect for the HC/E group was significant only under the VI schedule conditions, $F(3,56)=4.75$, $p<0.01$.

There was an overall trend for significant differences among groups, $F(3,8)=3.20$, $p<0.10$, but at each schedule/drug dose combination (except Baseline-2/VT schedule), a significant groups effect was found (all F's $(3,56)>4.20$, $p<0.01$). For the three lowest doses and for each schedule condition (except VT schedule at Baseline-2), the controls had fewer responses than the other lesion groups (all p's<0.05). At the two highest doses of L-PIA, the HC/E group had significantly fewer responses than the MS and HC/C groups ($p<0.05$), and at the highest L-PIA dose only the MS and HC/C groups had significantly more responses than controls (p's<0.01). A large difference between schedule conditions is apparent in Fig. 5, with the VI schedule producing more responses than the VT schedule, $F(1,8)=152.75$, $p<0.01$. A similar schedule-effect was found for all drug levels in the HC/C group (p's<0.01), for the three lowest drug levels in the MS group (p's<0.05), but only for the lowest L-PIA dose in the control and HC/E groups.

In summary, while all lesion groups displayed significantly higher response rates than controls at Baseline-2 and the lower L-PIA doses, the highest L-PIA dose reduced response rate in the HC/E group to a level not significantly different from controls.

L-PIA Dose-Effect Analysis—Activity

The two lower sections of Fig. 5 show the effect of various doses of L-PIA on activity in the several groups. The overall activity results here are similar to those previously described in the caffeine dose-effect section. Significant overall main effects were found for group, $F(3,8)=25.29$, $p<0.01$, and drug level, $F(3,24)=6.86$, $p<0.01$. At all dose levels of L-PIA and under each schedule condition, the HC/C group had significantly higher activity scores than all other groups (all p's<0.01). Further, significant dose-related decreases in the activity of HC/C animals were found under the VI, $F(3,56)=3.75$, $p<0.05$, and VT, $F(3,56)=6.78$, $p<0.01$, schedules. No significant differences in activity scores from the two schedule conditions were found.

In sum, the HC/C group displayed higher activity scores than all other groups (which did not differ from each other); this high level of activity was reduced in a dose-related manner by L-PIA but not to control levels of activity.

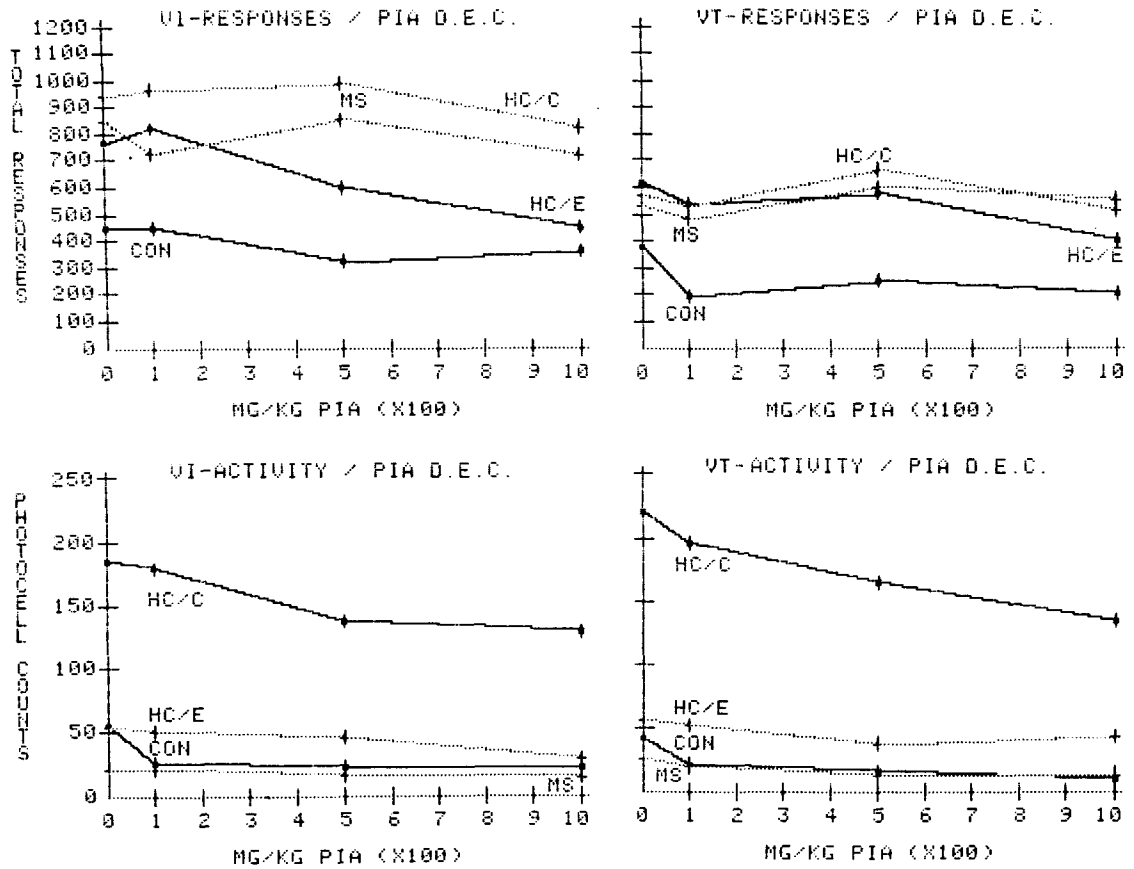

FIG. 5. Mean operant responses (top) and non-operant activity levels (bottom) per session during the VI (left) and VT (right) schedules after IP administration of L-PIA. Values=replicate means with N=3 (0=second baseline).

Combinations of Caffeine and L-PIA

Figure 6 shows the operant response activity data for Baseline-3, 0.1 mg/kg L-PIA + 32 mg/kg caffeine, and 32 mg/kg caffeine alone. The only significant group differences found were for Baseline-3 under both the VI, $F(3,40)=14.03$, $p<0.01$, and VT, $F(3,40)=3.09$, $p<0.05$, schedules and for the L-PIA + caffeine condition under the VI schedule, $F(3,40)=4.22$, $p<0.05$. The HC/C group had significantly higher response rates than controls at all the latter points ($p<0.01$) and higher rates than the HC/E group under the VI schedule for Baseline-3 and the L-PIA + caffeine condition ($p<0.01$ and $p<0.05$, respectively).

The MS group had higher responding rates than controls under the VI schedule for Baseline-3 and the L-PIA + caffeine condition ($p<0.01$ and $p<0.05$, respectively) and the HC/E group only had a significantly higher rate than controls at Baseline-3 under the VT schedule. There were significant main effects for both drug condition, $F(2,16)=22.82$, $p<0.01$, and schedule, $F(1,8)=34.65$, $p<0.01$. However, only the lesion groups displayed significant differences among drug conditions (all $F's(2,16)>6.5$, $p<0.01$). Only the MS and HC/C groups showed significant differences between schedule conditions, $F's(1,8)=12.95$ and 18.76, respectively, $p's<0.01$ with significantly more responses under the VI than under the VI schedule for all drug/baseline conditions (all $p's<0.05$). All lesion groups displayed significantly lower response rates under the two drug conditions than on Baseline-3 (all $p's<0.05$) under the VT schedule only, but only the MS and HC/C groups had significantly lower response rates on drug days than on the baseline under the VI schedule (all $p's<0.05$).

The results for the activity measure are displayed in the lower sections of Fig. 6. The only significant main effect is exclusively explained by the HC/C groups displaying significantly higher activity scores than all other groups at every drug/schedule condition (all $p's<0.01$).

In summary, all lesion groups displayed higher response rates than controls under the VI schedule. One and/or the other drug condition tended to return the elevated responding under the VI reinforcement schedule to control levels for the MS and HC/E groups. The two drug conditions also lowered responding in the HC/C group. As in the previous phases, the HC/C group displayed a persistently high level of operant chamber activity regardless of schedule or drug condition.

Baseline Comparisons

Figure 7 displays the response and activity measures for all groups at all four baseline conditions and under both schedules. For the response measure, all lesion groups displayed significantly higher response rates than controls on

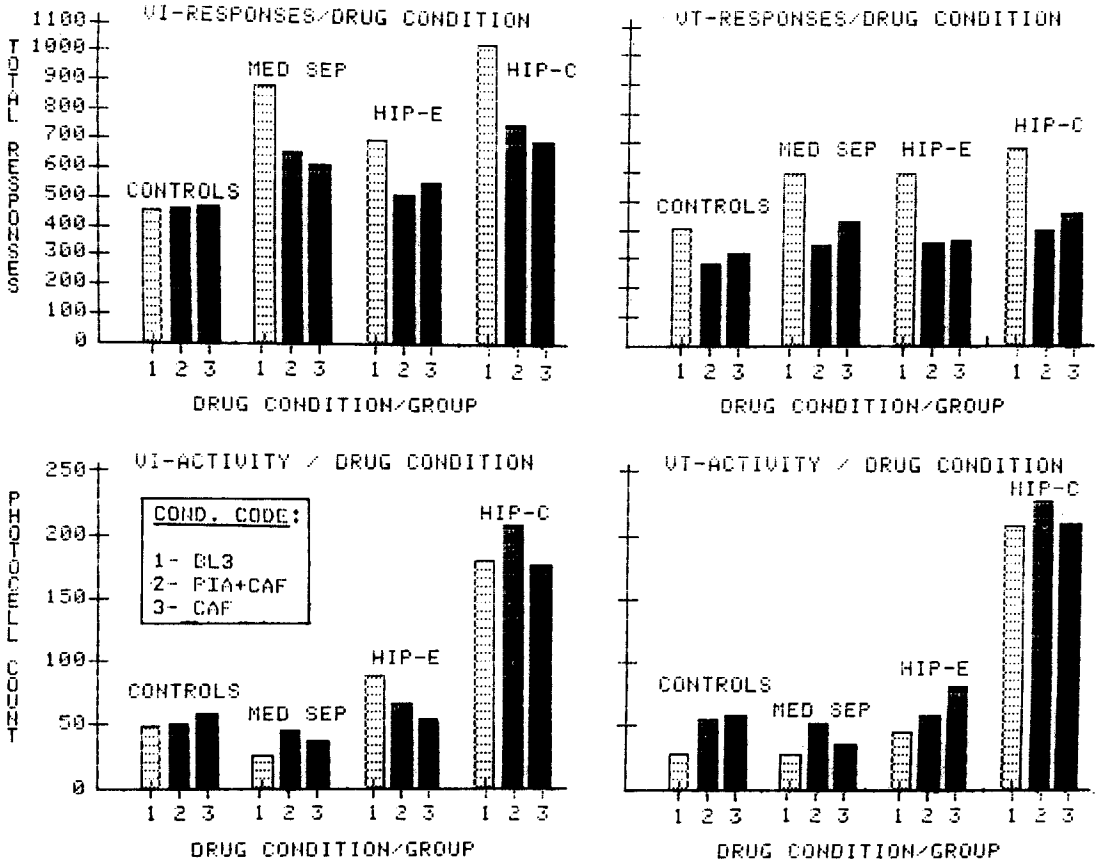

FIG. 6. The effects of 0.05 mg/kg L-PIA given concurrently with 32 mg/kg of caffeine [2] and 32 mg/kg of caffeine given alone [3] on both operant response (top) and non-operant activity (bottom) rates during both schedules are contrasted to each other and the third baseline [1]. Values represent replicate means per session (N=3). MED SEP=MS; HIP-E=HC/E; HIP-C=HC/C; CONTROLS=CON.

the first baseline under both schedule conditions (all F's(3,56)>5.4, p's<0.01) but significant group differences on the last three baselines were found only under the VI schedule (all F's(3,56)>3.9, p's<0.05). The MS and HC/E groups displayed decreases in response rate across baselines until they no longer were significantly different from controls under either schedule at the last baseline point. On the contrary, the HC/C group showed persistently high levels of responding across all baselines and had significantly higher response rates than all other groups on the last baseline under the VI schedule (all p's<0.05).

The activity measure yielded a significant main effect for groups, $F(3,8)=18.58$, $p<0.01$. The HC/C group had significantly higher activity scores than the other groups on Baseline-1 under both schedules (p's<0.01) and on Baseline-2 and Baseline-3 under the VT schedule. The HC/C group displayed a sharp decline in activity after the first baseline with activity scores on all subsequent baselines being significantly lower (all p's<0.05).

In summary, all lesion groups showed elevated response rates on Baseline-1 relative to controls, but the MS and HC/E groups displayed a decline in response rate after Baseline-1. Only the HC/C group displayed persistently high response rates across all baselines. Finally, the HC/C group displayed higher activity levels than the other groups. The latter hyperactivity was most notable on Baseline-1 but declined sharply on subsequent baseline days.

DISCUSSION

The results of the present experiment indicate that lesions of the medial septum (which do not produce initial periods of increased aggressiveness), lesions of the hippocampus (which leave portions of that structure intact), and lesions of the dentate granule cells (which do not directly destroy other parts of the hippocampus)—all produce rats which display significantly higher amounts of operant responding than non-lesioned control rats. During most of the treatments, these lesions generally produced animals which showed a reduction in operant behavior after a switch was made to the non-contingent schedule. The failure to produce the "superstitious" behavior (i.e., no reduction in operant rate with schedule changes from VI to VT) described by Devenport and Holloway [3] may have been due to our use of smaller hippocampal lesions or different schedule parameters. It is quite possible that the behavioral effect that they describe can only be produced in rats who have undergone complete hippocampal destruction as opposed to partial damage which might leave a sufficient amount of this neural area intact for the modulation of this behavior. This schedule difference in rate was not generally seen in the non-operant measure, thus indicating that this type of non-operant behavior is not greatly affected by changes in operant contingencies. Further research is needed to determine the extent and nature of this contingency insensitivity.

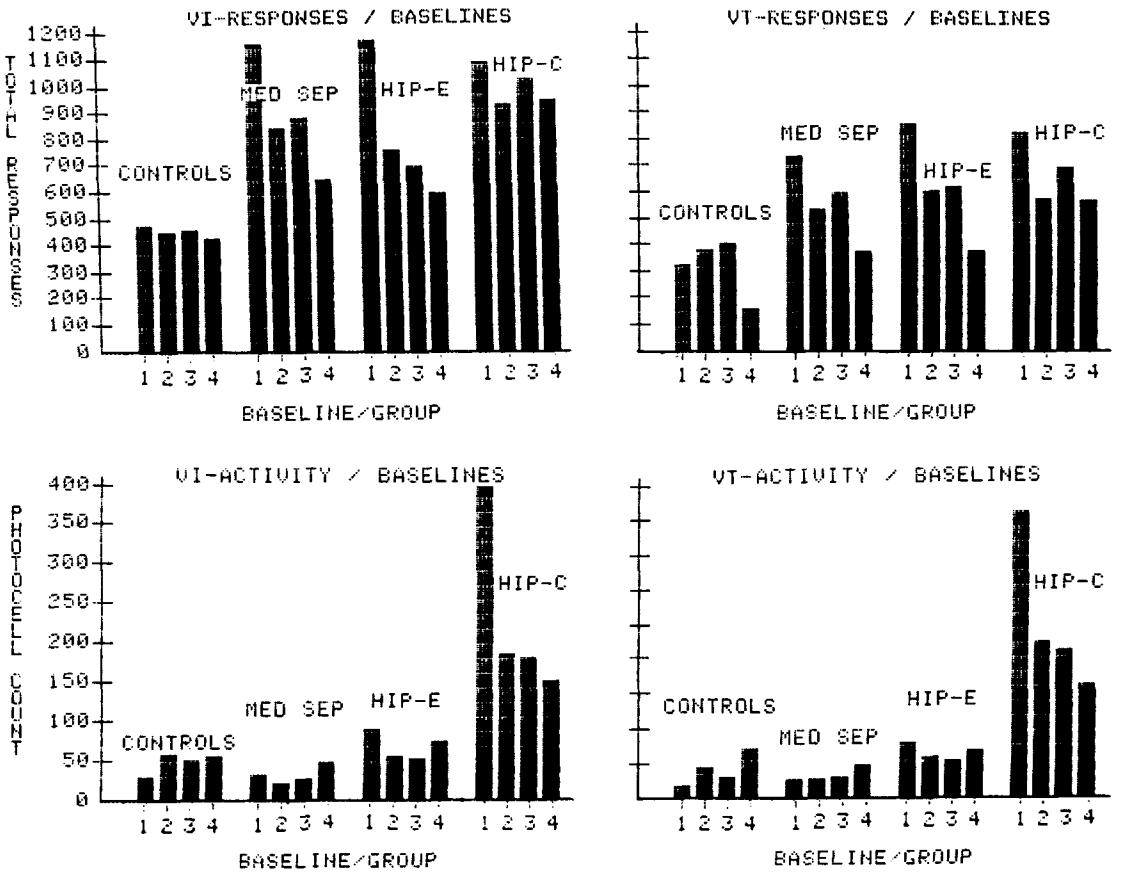

FIG. 7. The operant response (top) and non-operant activity (bottom) rates of all four groups at all four baseline treatments during both schedules. Values=replicate means per session (N=3), baselines numbered in order of temporal occurrence.

Nevertheless, the results of the present study are very similar to those described by Atnip and Hothersall [1] in rats with large lesions of the septum alone. This increase in response rate could very easily be viewed as either a lack of behavioral inhibition or as an increase in behavioral perseveration. In this regard, Jarrard [12] has shown that hippocampally lesioned rats produce significantly higher than normal rates of appetitively motivated operant response behavior on a variable interval schedule that does not appear to be due to either increased food motivation or pre-training rates of lever pressing.

It is clear that the response patterns of the lesioned animals were not efficient or adaptive relative to the control animals which received the same amount of reward for a smaller output of energy. Of course, one could argue that this behavior was somehow rewarding to the lesioned animal and thus "adaptive" in terms of that organism's objectives. Although this cannot be proved or disproved by the present results, it does fit in somewhat with Douglas' [5] idea of the hippocampus playing a role in the inhibition of emotional reactions. However, it should be noted that the MS group (which fits more into Douglas' first mode of hippocampal functioning, i.e., the medial septal-pyramidal theta generating system) and the HC/C group (which fits more into his second mode, i.e., the temporoammonic-dentate pathway) both generated the same initial pattern of operant behavior. In this regard, it is interesting to note that only the HC/C group produced levels of general non-operant activity during the experiment which were significantly higher than controls, making one wonder if this behavior might be more dependent on Douglas' second mode of functioning than was the operant behavior. Whatever the case, these data do confirm the existence of another type of behavioral response in which the medial septum, the dentate gyrus, and the hippocampus as a whole all play a role in the control of response "braking" in the aroused animal. This connection has been described previously for other behaviors [19].

The above data also indicate clearly that caffeine, in a dose-effective fashion, reduced the increased rates of operant responding seen in all three lesion groups. Caffeine, in doses equivalent to those used in the present experiment, has been reported to increase response rates under fixed-interval schedules [2]. There is evidence [20] to suggest that only very high doses of caffeine (i.e., 100 mg/kg) significantly reduce operant response rate on fixed-interval schedules. However, in the present situation caffeine did not significantly change the response rates of control animals in either direction. The reason for this possible discrepancy is unclear, but the caffeine insensitivity in controls may have been a function of the particular schedule parameters.

That all lesioned rats showed a caffeine dose-related decrease in responding could be partially due to some sensitization process produced by the several lesion procedures and reflected by a shift-to-the-left in the dose-effect curve.

The functional effect of such a shift was that 32 mg/kg of caffeine reduced the operant response rates of all three lesion groups to a level not significantly different from that of controls. Such a large rate reduction could indicate that caffeine is somehow producing an effect in the CNS which is at least behaviorally compensating for performance impairment produced by brain damage. Of course, this finding could merely be another example of the rate-dependent effect which is most often seen with more powerful CNS stimulants, such as methamphetamine [4]. If this is the case, then, at least in some instances, the phenomenon of rate-dependence might simply be another way that the CNS can be chemically modulated to compensate for the consequences of neural damage through, most likely, stimulation of those areas which modulate behavioral output.

One also observes a large drop in operant rate when comparing the first and second non-drug baselines for the MS and HC/E groups, especially during the VI schedule. This is very similar to a drop seen during the same period on the non-operant activity measure for the HC/C group during both schedules. In all cases, this rate reduction was temporally correlated with caffeine administration. Although the present study does not prove that caffeine is directly responsible for these baseline rate reductions, it seems highly unlikely that such marked behavioral changes are due solely to some time-dependent recovery of function mechanism since several weeks had elapsed between the actual surgeries and the reductions in question. It is interesting to note that such rate reductions were not seen after L-PIA administration but that smaller drops in the final baseline can be seen for some of these groups which were also temporally correlated with caffeine administration. Although we have no firm explanation for these results, it is possible that caffeine produces long-term, as well as short-term, reductions in various "hyperactive" behavior patterns. And this effect appears to be somewhat lesion-specific, since the HC/C group did not show such a substantial drop in operant rate, especially during the VI schedule. A similar paradigm might be useful in determining the ability of other CNS lesion types to alter behavioral output while also monitoring their resistance to pharmacological mediation or compensation.

At least two possible explanations come to mind which might fit the present data in regards to caffeine's possible mode of action. It could simply be that caffeine works in the present situation by operating through cyclic AMP and/or central catecholamines in a way that mimics some of the pharmacological actions of other CNS stimulants. On the other hand, it is also possible that caffeine is producing its effects by specifically blocking the adenosine receptor activity in various areas of the limbic system and basal ganglia, where the highest densities of these receptors are located in the rat [11], in a way which only behaviorally mimics the effects of other stimulants by perhaps reducing adenosine inhibition of catecholamine neurons. It is obvious that L-PIA administration produced effects, especially during the VI schedule, which were both similar and different to those effects seen with caffeine. Specifically, neither the MS nor the HC/C group showed significant dose-effect operant rate reduction with L-PIA while the HC/E group did. This would seem to indicate that specific activation of adenosine receptors by L-PIA at the doses tested produced effects similar to caffeine only in rats with hippocampal lesions which did not virtually destroy either the medial septum or the dentate gyrus. In the control animals, both drugs appeared to produce the same small non-significant operant rate effects. L-PIA also produced some small reductions in non-operant activity in the HC/C group, which was surprising in that this drug reduced the home-cage activity levels of all subjects in a dose-effective manner to the point of virtual immobility and loss of postural support at the highest dose for some members of all the groups. Although dramatic, this effect was almost totally reversed upon introduction to the testing apparatus.

Since L-PIA did not produce the same effects as caffeine, at least in the operant measure in the MS and HC/C groups and assuming that caffeine produced its effects by direct adenosine receptor modulation, one might speculate that L-PIA could be used to block the effect of caffeine seen in the MS and HC/C groups or perhaps even produce a syngergistic effect with it in the HC/E group. But the present results indicate that L-PIA produced neither of these results in that the combination of both drugs did not produce an effect significantly different from caffeine alone. It is possible that caffeine produced its behavioral effects in this experiment by modulating some neurochemical system outside of those adenosine receptors assumed to be directly affected by L-PIA. Since only one dose of each drug was tested in combination, however, it is possible that other dose combinations would prove more effective in this regard or at least produce a different effect from the one described above. More research is needed in this area, but whatever the mechanism of caffeine's actions, it cannot depend on the viability of either the medial septum or the dentate gyrus, since these effects were seen in animals without these specific structures intact.

Some recent evidence suggests that changes in adenosine receptor activity might be most important for the functioning of the pyramidal cells of Ammon's horn, most specifically the CA1 area [15]. Of course, since these drug effects were generally seen only in those animals with damage to some part of the hippocampal system, the most likely site of action could be outside of this system in some area, such as the striatum, which might be responsible for the control of behavior which is also at least indirectly modulated by the hippocampal system. In this regard, it has been demonstrated that caffeine enhances the rotational behavior of rats produced by dopamine receptor stimulating agents, perhaps by stimulating the release of dopamine in the striatum [7].

The present data are also interesting in light of their possible application to particular clinical situations. Specifically, it appears as if well localized lesions of the dentate granule cells produce animals which show increased levels of both operant and non-operant activity and that caffeine reduces these levels of activity to more normal levels. It has been demonstrated before that caffeine produces a varying amount of improvement on rating scales of hyperactivity in childhood hyperactivity or MBD cases [8]. It also has been suggested that interference with hippocampal functioning, especially in regards to dentate granule cells which are thought to be at least partially responsible for behavioral "maturation," could be an underlying physiological cause for this clinical problem [19]. With the above in mind, it is possible that the intracerebral application of colchicine in rats produces an animal model of at least one form of childhood hyperactivity, an idea we are presently investigating.

REFERENCES

1. Atnip, G. and D. Hothersall. Response suppression in normal and septal rats. *Physiol Behav* **15:** 417–421, 1975.
2. Carney, J. M. Effects of caffeine, theophylline and theobromine on schedule-controlled responding in rats. *Br J Pharmacol* **75:** 451–454, 1982.
3. Devenport, L. D. and F. A. Holloway. The rat's resistance to superstition: Role of the hippocampus. *J Comp Physiol Psychol* **94:** 691–705, 1980.
4. Dews, P. B. Studies on behavior. IV. Stimulant actions of methamphetamine. *J Pharmacol Exp Ther* **122:** 137–147, 1958.
5. Douglas, R. J. The development of hippocampal function: Implications for theory and for therapy. In: *The Hippocampus*, edited by R. L. Isaacson and K. H. Pribram. New York: Plenum Press, 1975, pp. 327–361.
6. Finger, S. and D. G. Stein. *Brain Damage and Recovery: Research and Clinical Perspectives*. New York: Academic Press, 1982, pp. 227–256.
7. Fredholm, B. B., M. Herrera-Marschitz, B. Jonzon, K. Lindstrom and U. Ungerstedt. On the mechanism by which methylxanthines enhance apomorphine induced rotation behavior in the rat. *Pharmacol Biochem Behav* **19:** 535–541, 1983.
8. Garfinkel, B. D., C. D. Webster and L. Sloman. Response to methylphenidate and varied doses of caffeine in children with attention deficit disorder. *Can J Psychiatry* **26:** 395–401, 1981.
9. Goldschmidt, R. B. and O. Steward. Preferential neurotoxicity of colchicine for granule cells of the dentate gyrus of the adult rat. *Proc Natl Acad Sci USA* **77:** 3047–3051, 1980.
10. Goodman, L. S. and A. Gilman. *The Pharmacological Basis of Therapeutics*. New York: MacMillan Publishing Co., 1980.
11. Goodman, R. R. and S. H. Snyder. Autoradiographic localization of adenosine receptors in rat brain using (3H) cyclohexyladenosine. *J Neurosci* **2:** 1230–1241, 1982.
12. Jarrard, L. E. Hippocampal ablation and operant behavior in the rat. *Psychon Sci* **2:** 115–116, 1965.
13. Kimble, D. P. Hippocampus and internal inhibition. *Psychol Bull* **70:** 285–295, 1968.
14. Konig, J. F. R. and R. A. Klippel. *The Rat Brain*. Baltimore: Williams and Wilkins, 1963.
15. Lee, P. S., P. Shubert, M. Reddington and G. W. Kreutsberg. Adenosine receptor density and the depression of evoked neuronal activity in the rat hippocampus in vitro. *Neurosci Lett* **37:** 81–85, 1983.
16. Luria, A. R. Disorders of "simultaneous perception" in a case of bilateral occipito-parietal brain injury. *Brain* **82:** 437–449, 1959.
17. Modrow, H. E., F. A. Holloway, H. D. Christensen and J. M. Carney. Relationship between caffeine discrimination and caffeine plasma levels. *Pharmacol Biochem Behav* **15:** 323–325, 1981.
18. Snyder, S. H., J. J. Katims, Z. Annan, R. F. Bruns and J. W. Daly. Adenosine receptors and behavioral actions of methylxanthines. *Proc Natl Acad Sci USA* **78:** 3260–3264, 1981.
19. Wallace, R. B., R. Kaplan and J. Werboff. Hippocampus and behavioral maturation. *Int J Neurosci* **7:** 185–200, 1977.
20. Wayner, M. J., F. B. Jolicoeur, D. B. Rondeau and F. C. Barone. Effects of acute and chronic administration of caffeine on schedule dependent and schedule induced behavior. *Pharmacol Biochem Behav* **5:** 343–348, 1976.
21. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1971, p. 545.