Effect of ^{125}I gG-saporin injections into the nucleus basalis magnocellularis on acquisition and performance of a go/no-go procedure in the rat

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The effects of injections of low (105 ng) and high (210 ng) doses of the cholinergic-selective neurotoxin ^{125}I gG-saporin (SAP) into the nucleus basalis magnocellularis on both the acquisition and performance of a go/no-go operant task in Long-Evans rats were examined and were correlated with regional changes in choline acetyltransferase (ChAT) activity. The go/no-go procedure rewards a rat for pressing a lever on alternate trials, with intertrial intervals (i.e., delays) of 5, 20, and 40 sec. Over a period of 15 days, normal rats rapidly become proficient in this task, so that the ratio of latency to the first press on the rewarded go trial to latency to press on the nonrewarded no-go trial is significantly less than 1. With injections made prior to acquisition, there was a significant disruptive effect of both doses of SAP that was not delay dependent. In previously trained rats, SAP disrupted performance during a 2-week training period, with the high dose of SAP having a more pronounced effect. A uniform 60% reduction of cortical ChAT activity relative to control was found after either high- or low-SAP injections; however, there was a dose-dependent reduction in hippocampal ChAT activity with SAP treatment (low dose [20%–29%] and high dose [39%–44%]). Thus, the dose-dependent disruption in rats trained prior to injection may be related to combined cortical and hippocampal cholinergic deficits. The presence of an impairment at even the shortest delay suggests that impaired working memory may play only a minor role in the go/no-go deficit produced by disruption of the basal forebrain cholinergic system and that impaired attention may be more important.

The effects of basal forebrain lesions on learning and memory and on attentional processes in the rat have been well documented over the past years (Baxter, Bucci, Gorman, Wiley, & Gallagher, 1995; El-Defrawy et al., 1985; Hepler, Olton, Wenk, & Coyle, 1985; McGaughy, Kaiser, & Sarter, 1996; Muir, Dunnett, Robbins, & Everitt, 1992; Muir, Everitt, & Robbins, 1994, 1995; Olton, Wenk, Church, & Meck, 1988; Robbins, Everitt, Marston, et al., 1989; Robbins, Everitt, Ryan, et al., 1989; Voytko et al., 1994; Wenk, Stoehr, Quintana, Mobley, & Wiley, 1994). Early work using nonselective neurotoxins such as ibotenic acid suggested an involvement of the basal forebrain in learning and memory. Cholinergic neurons within the nucleus basalis magnocellularis (NBM) are the predominate source of cholinergic input to the amygdala and much of the cerebral cortex, whereas cholinergic neurons in the medial septal/diagonal band area of the basal forebrain supply cholinergic innervation to the hippocampus and cingulate cortex (McKinney,

Coyle, & Hedreen, 1983). Thus, the observation of impaired learning and memory performance following ibotenic acid lesions of these basal forebrain regions was interpreted as being support for the role of the forebrain cholinergic system in learning and memory. However, subsequent work demonstrated that NBM injections of more selective neurotoxins, such as quisqualic acid, had little or no effect on memory, but rather, a more specific effect on attentional processing (Dunnett, Everitt, & Robbins, 1991; Muir et al., 1994; Robbins, Everitt, Marston, et al., 1989; Wenk, 1997).

More recently, a neurotoxin, ^{125}I gG-saporin (SAP), which selectively targets basal forebrain cholinergic neurons (Heckers et al., 1994; Torres et al., 1994; Waite et al., 1994), has been shown to reduce cortical choline acetyltransferase (ChAT) activity (Baxter et al., 1995) and cortical AChE-positive fiber density (McGaughy et al., 1996) when micro-injected into the NBM. Site-specific NBM SAP injections have little or no effect on the acquisition of spatial learning (Baxter et al., 1995; Berger-Sweeney et al., 1994; Torres et al., 1994). This is in contrast to the impairments in learning and memory task performance following i.c.v. SAP treatment, which induces a more generalized loss of cholinergic markers in both the cortex and the hippocampus, as well as some damage to non-cholinergic neurons in the cerebellum (Berger-Sweeney et al., 1994; Nilsson et al., 1992).

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In contrast to the largely negative results obtained with spatial learning and memory tasks, tasks assessing attention are consistently impaired by SAP lesions of the basal forebrain (Chiba, Bucci, Holland, & Gallagher, 1995; Han, Gallagher, & Holland, 1995; McGaughy et al., 1996; Stoehr & Wenk, 1995). Attention has been conceptualized as including vigilance, arousal, expectancy, and the ability to discriminate stimuli (Wenk, 1997). It is probably involved in the acquisition of most operant tasks in which animals are required to respond to a stimulus.

The behavioral procedures used to measure attention in rats (Baxter et al., 1995; Chiba et al., 1995; Holley, Turchi, Apple, & Sarter, 1995; McGaughy et al., 1996; Muir, Dunnett, et al., 1992; Muir et al., 1995; Muir, Robbins, & Everitt, 1992; Pang, Williams, Egeth, & Olton, 1993; Robbins, Everitt, Marston, et al., 1989; Stoehr & Wenk, 1995) typically involve visual and/or auditory processing by the animal. These procedures usually involve long training periods, often up to 6 months, in operant chambers. In contrast, the go/no-go procedure (Winocur, 1985) is a simple operant procedure for rats that requires only 10–15 sessions for the rat to become proficient in the task. This task makes minimal demands on visual and auditory acuity. In addition, it has been proposed that this task assesses both attentional and memory processes, depending on the length of the delays between trials (Winocur, 1985). For example, electrolytic lesions of the hippocampus prior to acquisition of this task produced no disruption of performance with delays shorter than 10 sec but produced deficits at delays longer than 10 sec, which were attributed to memory loss in these animals; in contrast, thalamic lesions impaired performance at both long and short delays, a pattern consistent with impaired attention (Winocur, 1985).

In the current study, we investigated the effects of NBM injections of SAP on go/no-go performance. SAP was injected either prior to or following training in this go/no-go procedure to investigate whether a cholinergic depletion would affect the acquisition and/or the performance of this task. If this simpler procedure taps forebrain cholinergic mechanisms involved in attention (shorter delays), SAP injections into the NBM would result in an overall disruption of performance in this task; however, if these cholinergic mechanisms are involved in memory (longer delays), SAP injections into the NBM would result in a delay-dependent disruption of performance in this task. Because SAP injections into the NBM can also produce some disruption of cholinergic input to the hippocampus (Berger-Sweeney et al., 1994; Wenk et al., 1994), two doses of SAP were utilized, and ChAT activity in both the cortex and the hippocampus was assessed following behavioral testing.

METHOD

Subjects

Sixty-four male Long-Evans rats (CrI:(LE)BR) obtained from Charles River Labs (Portage, MI), weighing 225–250 g, were used. They were housed 1/cage and placed on a restricted diet of 18–20 g of Purina rat chow, with water being available ad lib. The room was

illuminated on a 14:10-h light:dark cycle. All testing was conducted during the light phase of the cycle.

Apparatus

The apparatus consisted of eight identical modular test cages (Coulbourn Instruments Inc., Allentown, PA) equipped with a retractable lever, a 45-mg food pellet feeder with a central hopper, and a houselight, each enclosed in a ventilated isolation cubicle. The levers, lights, and timing were controlled by Habitest interface and L2T2 software using a Compaq 450 XE computer.

Lever Training Procedure

The rats were placed on a restricted diet for 3 days before beginning training. Lever training began with daily 60-min sessions in the operant chambers in which each leverpress was reinforced with a food pellet. Rats were trained to a criterion of 75 responses in 20 min for 2 consecutive days. The rats were then divided into two equal groups, a performance group (PERF), which was trained for 3 weeks in the go/no-go procedure before receiving SAP injections into the NBM and then retested, and an acquisition group (ACQ), which received SAP injections prior to 3 weeks of go/no-go training.

Go/No-Go Procedure

The rat was placed in a dark (no houselight) chamber with the lever retracted. The session, signaled by turning on the houselight and presenting the lever, started 5 min later. The rat was always reinforced for leverpresses on the first lever presentation (a go trial). Trials were 20 sec in duration, during which, for go trials only, the rat received pellets on a continuous reinforcement (CRF) schedule. The lever was then withdrawn and the houselight extinguished. After a delay, the next trial was a no-go trial, during which the lever was presented and the houselight was on but the rat was not reinforced for leverpressing. Alternating go and no-go trials were presented throughout the session. Trials were separated by delay periods of either 5, 20, or 40 sec, during which the houselight was extinguished. Delays were varied randomly, with the constraint that 10 of each delay interval preceded go trials and 10 of each delay interval preceded no-go trials. The rat was reinforced for the first barpress and for each subsequent press during the go trial and not rewarded during the no-go trial. The mean latency to the first barpress for each of the trials at each delay was used to calculate the performance ratio—that is, (the mean latency to press on the go trials)/(the mean latency to press on the no-go trials). Separate performance ratios were calculated for each delay interval. Daily sessions were conducted Monday through Friday of each week.

Surgery

The rats were anesthetized with sodium pentobarbital (55 mg/kg i.p.). They were placed into a Kopf student stereotaxic instrument and bilaterally injected into the NBM via a 30-gauge micro injection cannula, with either 210 or 105 ng of ¹⁹²IgG SAP, in 0.5 μ l phosphate buffered saline (PBS) over a period of 150 sec. Control rats were injected with 0.5 μ l of phosphate buffered saline (PBS). The injectors were left in place for an additional 60 sec following injection before removal. Stereotaxic coordinates (from bregma) for the injections were AP -0.6 mm, ± 2.8 mm lateral, and -7.5 mm below the skull surface. Animals were allowed a minimum of 10 days to recover following surgery. CRF performance was reinstated prior to initiating go/no-go testing.

Biochemical and Histological Evaluation

Following testing, the rats were sacrificed, the brain removed, and the hippocampus and cortex dissected out and frozen for analysis of ChAT activity by an adaptation (Decker, Majchrzak, & Anderson, 1992) of the procedure of Fonnum (Fonnum, 1975). The forebrains of a representative subset of PBS control and high-dose SAP were saved in 10% formalin and histologically examined.

Figure 1A. Rapid acquisition of the go/no-go procedure by intact rats over 3 weeks of training. The performance ratio is calculated as (latency to press the lever on the go trial)/(latency to press the lever on the no-go trial).

There was no evidence of gross physical damage at the injection site under microscopic examination.

Data Analysis

The latency to respond and mean performance ratio for each of the intervals for each week of training for go and no-go trials were analyzed with a repeated measures analysis of variance (ANOVA). Comparisons of individual delay intervals were performed where appropriate with subsequent post hoc analysis (Fisher least significant difference). A one-way ANOVA was used for the analysis of ChAT levels.

RESULTS

Acquisition of the Go/No-Go Procedure in Noninjected Rats

The rapid acquisition of the go/no-go procedure by the PERF group prior to surgery can be seen in Figure 1A. During the 1st week, the animals performed at chance levels, and it was not until the end of the 2nd week of training that the rats showed a separation in the performance ratio between the short and the long delay intervals. During the 3rd week of training, there was no overall significant increase in performance over days [$F(4,240) = 1.472$, $p = .2148$]; however, there was a significant delay effect [$F(2,60) = 31.659$, $p = .0001$]. The days $\times$ delays interaction approached significance [$F(4,8) = 1.808$, $p = .0762$]. The rats performed significantly more poorly at the 40-sec delay, relative to the shorter delays.

These effects are also evident when the response latencies are examined (Figure 1B). The rats start by responding 5–6 sec following the lever presentation, and, as they learn the task, the response latency decreases over weeks for go trials and increases for no-go trials (as is shown by the week $\times$ go/no-go interactions [$F(2,60) = 96.463$, $p < .0001$]). This effect was more pronounced at the two

shorter delays (week $\times$ delay $\times$ go/no-go interaction [$F(4,120) = 13.076$, $p < .0001$]). During the 3rd week, there was no significant effect of delay on go trial latency [$F(2,62) = 2.69$, $p = .08$], but there was a significant effect of delay on no-go trial latencies in the 3rd week [$F(2,62) = 65.306$, $p = .0001$], with all delays being different from each other ($p < .005$).

Performance Following a SAP Injection Into the NBM

The effect on performance in the 1st and 2nd weeks of training in the PERF group following the injection of SAP into the NBM can be seen in Figure 2A. Analysis with a repeated measures ANOVA, with SAP as a between-measures factor and weeks and days and delays as within-measures factors, revealed significant SAP [$F(2,28) = 7.02$, $p = .003$], weeks [$F(1,28) = 0.729$, $p = .006$], and delay [$F(2,56) = 19.405$, $p = .0001$] effects but no days effect [$F(4,112) = 0.548$, $p = .7$]. There was no interaction between days, delay, weeks, or SAP. The averages for each week were, therefore, collapsed across days and analyzed separately. For the 1st week, there was an overall significant effect of SAP [$F(2,56) = 7.012$, $p = .0034$] and delay [$F(2,56) = 7.610$, $p = .0012$], with no delay $\times$ SAP interaction [$F(4,56) = 0.798$, $p = .5318$]. Post hoc analysis showed that only the high-dose SAP group was significantly impaired relative to the PBS controls. During the 2nd week of testing, there was a general trend for improved performance in all groups, but there were still significant effects of SAP [$F(2,56) = 4.903$, $p = .0149$] and delays [$F(2,56) = 11.406$, $p = .0001$], with no delay $\times$ SAP interaction [$F(4,56) = 0.785$, $p = .5395$].

The lesion effect during the 1st week was the result of increased latencies on go trials [$F(2,29) = 3.501$, $p = .0434$] that did not interact with delay [$F(4,58) = 1.4$, $p = .245$]

Figure 1B. Changes in latency to press the lever during acquisition of the go/no-go procedure at the different delay periods over 3 weeks of training.

and decreased latencies on no-go trials that were restricted to the short delay (overall lesion effect, $[F(2,29) = 1.128, p = .3374]$; delay $\times$ lesion interaction, $[F(4,58) = 9.319, p = .0001]$), with a significant effect for both low- ($p = .035$) and high- ($p = .0005$) SAP groups. During the 2nd week, there was no treatment effect on either the go $[F(2,29) = 0.799, p = .46]$ or the no-go $[F(2,29) = 0.888, p = .423]$ latencies, although the delay $\times$ lesion interaction approached significance $[F(4,58) = 2.338, p = .066]$ for no-go trials. These data are shown in Figure 2B.

The Effect of a SAP Injection Into the NBM on Acquisition of the Go/No-Go Procedure

Given that rats trained on the go/no-go procedure prior to surgery did not perform at better than chance levels until the 3rd week of training (Figure 1), performance during the 3rd week of training was used to evaluate acquisition in rats trained after surgery. The effect of the SAP injection into the NBM on acquisition of the go/no-go procedure can be seen in Figure 3A. A repeated measures analysis, with SAP as a between-groups measure and day and delay as within-groups measures, revealed a significant SAP effect $[F(2,232) = 5.220, p = .0116]$. There was also a significant effect of day $[F(4,232) = 3.929, p = .005]$, as well as a significant effect of delay $[F(2,232) = 8.882, p = .0004]$. The day effect was the result of chance performance in even the PBS control rats on Monday but not on subsequent days. When the data were reanalyzed for the last 4 days (Tuesday through Friday), there was a significant effect of SAP $[F(2,174) = 6.669, p = .0041]$ and a significant effect of delay $[F(2,174) = 4.652, p = .0134]$. There was no significant day effect $[F(3,174) = 2.256, p = .0875]$ or SAP $\times$ delay interaction $[F(4,174) = 0.874, p = .5]$ (Figure 3B). Both the low- and the high-SAP groups were significantly ($p < .01$) different from control.

The latency data for Days 12–15 are shown in Figure 3C. There was no significant lesion effect on either the go $[F(2,29) = 1.68, p = .204]$ or the no-go $[F(2,29) = 1.126, p = .338]$ trials; however, there was a delay $\times$ lesion effect $[F(4,58) = 3.88, p = .0073]$ on no-go trials. This reflects a significant lesion effect on no-go trials $[F(2,29) = 3.504, p = .043]$ at the 5-sec delay, with the SAP High group significantly different from control ($p = .016$) and no lesion effect at the longer delays.

ChAT Analysis

Biochemical (ChAT) analysis of the frontal cortex and the hippocampus in both the PERF and the ACQ groups shows a similar reduction by SAP treatment (Figure 4). There was a significant reduction in ChAT levels in the cortex $[F(2,28) = 39.371, p = .0001]$; Figure 4A) and in the hippocampus $[F(2,28) = 9.611, p = .0007]$ in the PERF group, with both SAP groups being significantly different from controls. Cortical and hippocampal ChAT reduction in the ACQ group (Figure 4B) was also significant [cortex, $F(2,29) = 68.739, p = .0001$; hippocampus, $F(2,29) = 15.09, p = .0001$], with both doses of drug being significantly different from control on post hoc analysis. The cortical ChAT depletion for both doses of SAP in the cortex was equivalent. In the hippocampus, the decrease was dose dependent for both groups of rats. In the performance group, there was a significant difference ($p < .05$) between the low- and the high-dose SAP groups, and, in the acquisition group, the difference approached significance ($p = .08$).

DISCUSSION

With daily training sessions, acquisition of the go/no-go procedure became apparent toward the end of the 2nd

Figure 2A. The performance group rats; changes in performance ratio at different delay periods for 2 weeks following saporin (SAP) injection into the nucleus basalis magnocellularis.

week of training, and it was during the 3rd week that all the rats became proficient and stable in performance. This is similar to the data obtained by Winocur (1985), where, on the 12th day of training, the rats were proficient in this task. The rats' latencies to respond on all trials initially was about 6 sec. As they learned to discriminate between the go and the no-go trials, they increased their latencies to respond on no-go trials and decreased their latencies to respond on go trials. By the 3rd week of training, their latencies to respond on go trials had decreased to approximately 3 sec, regardless of the length of the delay, whereas their latencies on no-go trials were delay

dependent. In the case of the 40-sec delay, the rats tended to respond as if all trials were go trials.

In rats trained prior to injection, the SAP groups performed significantly worse during the 1st week of training after the injection. The nature of the lesion effect was somewhat different in the two lesion groups during this 1st week. The deficit in the low-dose group appeared to be due to a decrease in latencies on no-go trials that was most pronounced at the short delay. In contrast, the deficit in the high-dose group was due to both a decrease in no-go trial latencies and an increase in go trial latencies. Both effects were more prominent at the short delay. Thus,

Figure 2B. Changes in latency to press the lever on go and no-go trials for the performance group rats at different delay periods for 2 weeks following saporin (SAP) injection into the nucleus basalis magnocellularis.

the high-dose lesion appeared to revert the animals back to an earlier stage of training, in that the SAP High latencies for both go and no-go trials returned to between 5.5 and 6.5 sec. However, in the 2nd week, this effect was not evident, as the high-dose lesion effect appeared to be related mainly to changes in the no-go trial latencies.

In the ACQ group, there was a significant overall effect of the SAP on the acquisition of the task. At the two shortest delays, both the low- and the high-SAP groups showed a performance deficit. At the 40-sec delay, all the animals were performing at chance; thus, an interpretation of SAP effects at the 40-sec delay cannot be made. The performance at the 5-sec delay in the control rats in the ACQ group was not different from that for the rats in the PERF group at this delay. Although there was a tendency for the SAP-treated rats to respond more slowly on go trials, this was not statistically significant, so the impairment appeared to be related primarily to decreases in no-go trial latencies, particularly at the short delay. The fact that the SAP-injected animals were unable to per-

form at the 5-sec delay may be attributed to disruption of attentional processes important for acquisition of this task. It would follow that, if the rats are unable to acquire the task at the shortest delay, they would also have a similar deficit at the longer delay, which is the case here. Another consideration might be that there is an effect on procedural learning in the SAP-lesioned rats. However, in other studies, following SAP lesions to the NBM, rats were able to acquire procedures such as the Morris water maze and the radial arm maze (Dornan et al., 1996).

The data from the ChAT analysis may have some bearing on these data. In the cortex, ChAT levels were decreased about 60% in both the low- and the high-SAP groups, whereas in the hippocampus, there was a significant dose-dependent decrease of approximately 25% in the low-SAP and 40% in the high-SAP groups. This is consistent with previous reports of mild to moderate loss of septohippocampal cholinergic neurons with NBM injections of SAP (Berger-Sweeney et al., 1994; Heckers et al., 1994; Wenk et al., 1994). These results emphasize

Figure 3. (A) Performance in the 3rd week of acquisition for the acquisition group following the saporin (SAP) injection into the nucleus basalis magnocellularis. Performance ratios displayed across delays. (B) Performance for the acquisition group rats for the last 4 days of training.

the importance of assessing both cortical and hippocampal ChAT activity after SAP injections into the NBM. Moreover, these findings suggest that disruption of hippocampal cholinergic function may play a role in some of the effects of NBM SAP injections reported in the literature, inasmuch as lesion effects on hippocampal ChAT activity are not always evaluated.

Inasmuch as, in the current study, the two doses of SAP produced comparable disruption of cortical cholinergic input but different degrees of disruption of hippocampal cholinergic input, differential behavioral effects of high and low doses of SAP could be related to hippocampal cholinergic depletion. Given the larger depletion of hip-

pocampal ChAT activity in the high-SAP group, it is tempting to suggest that the go/no-go deficits we observed were due to disruption of hippocampal function. However, in a previous study in this laboratory (unpublished results), a medial septal SAP injection, which reduces hippocampal ChAT activity, did not affect the performance of rats previously trained in the go/no-go procedure. It is possible, however, that the dose-dependent disruption of go/no-go performance obtained in the present study resulted from a combined disruption of hippocampal and cortical functions. Combined SAP injections into the NBM and septum have been shown to produce modest deficits in radial arm maze performance that were not

observed with independent injections into either structure (Dornan et al., 1996). In this same study, combined lesions did not disrupt water maze performance. These data, combined with the present observations, suggest that concurrent disruptions of cortical and hippocampal cholinergic functions may have greater impact on performance. Alternatively, damage to cholinergic striatal interneurons sometimes observed after NBM injections of SAP (Heckers et al., 1994) could play a role.

In the Winocur study (1985), a hippocampal lesion produced a decrease in performance at the longest delays but not at the short delays, which was interpreted as a decrease in short-term memory. In the present study, most effects of the SAP lesions were observed at even the shortest delay. Thus, it is not likely that the effects we observed were primarily on working memory. Effects on attention or on reference memory are more likely. It is unlikely that effects on motor performance, or other factors that might produce nonspecific disruption of the ability of the animals to perform the task, would explain these effects, because response latencies on go trials were relatively stable or only transiently affected.

Interestingly, some of the effects of SAP lesions were actually more pronounced at the short delay than at longer delays. Although this could be due to floor effects related to the relatively poorer performance of control rats at the longer delay, it is also possible that the lesion may have had an effect on the ability of the animals to discriminate between trial types when they were presented closely together. In an observation that may be related, we have noted that normal animals have greater difficulty learning

Figure 3C. Latency to press the lever during go and no-go trials for the acquisition group for the last 4 days of training.

ChAT LEVEL REDUCTION

Figure 4. ChAT levels in the performance group (A) and in the acquisition group (B) show a significant ($p < .01$) reduction in both saporin (SAP) High and SAP Low groups in the hippocampus and cortex. In the hippocampus, there is a significant difference between the low- and the high-SAP groups ($p < .05$) in the performance group and a nearly significant effect in the acquisition group ($p = .08$).

to discriminate between go and no-go trials when the delay is in the range of 1–2 sec than when it is in the range of 5–10 sec (unpublished observation).

In summary, low- and high-dose SAP injections into the NBM were shown to produce equivalent reductions of cortical ChAT but dose-dependent reductions of hippocampal ChAT. The injections produced deficits in both the acquisition and the performance of a go/no-go operant procedure in the rat. SAP injections impaired acquisition on performance of the task, and the high-dose SAP injection produced a more pronounced impairment. Impairments at even the shortest delays in rats trained after SAP

injections were observed, which is consistent with the idea that the disruption of the basal forebrain cholinergic system may have greater impact on attentional processes involved in encoding.

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