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RC-3095, a bombesin/gastrin-releasing peptide receptor antagonist, impairs aversive but not recognition memory in rats

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Abstract

Bombesin and its mammalian equivalent, gastrin-releasing peptide (GRP), stimulate cell proliferation and are involved in the pathogenesis of several types of human cancer. Bombesin-like peptides also display neuroendocrine activities and regulate neural function. In the present study, we evaluated the effects of the bombesin/GRP receptor antagonist (D-Tpi^6, Leu^{13} psi[CH_2NH]-Leu^{14}) bombesin-(6–14) (RC-3095), an experimental antitumor drug, on memory in rats. Adult female Wistar rats were treated with an intraperitoneal injection of RC-3095 (0.2, 1.0 or 5.0 mg/kg) 30 min before training in either inhibitory avoidance or novel object recognition tasks. Retention test trials were carried out 1.5 (short-term memory) or 24 h (long-term memory) after training. RC-3095 at the doses of 0.2 or 1.0 mg/kg, but not at the dose of 5.0 mg/kg, impaired both short- and long-term inhibitory avoidance retention, but did not affect recognition memory. The memory-impairing effect of RC-3095 could not be attributed to alterations in sensorimotor functions. The results show that the antitumor drug/GRP antagonist RC-3095 impairs formation of aversive memory.

Keywords: RC-3095; Bombesin; Gastrin-releasing peptide; Anticancer drug; Behavior; Memory

1. Introduction

Bombesin-like peptides such as the gastrin-releasing peptide (GRP) stimulate cell proliferation and are involved in the pathogenesis of human lung, pancreatic, prostatic, ovarian, and breast cancers and glioblastomas (Bologna et al., 1989; Moody and Cuttitta, 1993; Siegfried et al., 1994; Wang et al., 1996; Chatzistamou et al., 2001). Bombesin/GRP receptor antagonists such as (D-Tpi^6, Leu^{13} psi[CH_2NH]-Leu^{14}) bombesin-(6–14) (RC-3095) have been developed and proposed as potential antitumor agents (Qin et al., 1994; Szepeshazi et al., 1997; Chatzistamou et al., 2001).

Bombesin/GRP receptors and bombesin-like peptides are widely distributed in the mammalian brain, and bombesin-like peptide receptor signaling pathways are involved in several aspects of brain function, regulating feeding, satiety, aversion, reward, anxiety, as well as learning and memory processes (Flood and Morley, 1988; McCoy and Avery, 1990; Morley et al., 1992; Williams and McGaugh, 1994; Wada et al., 1998; Santo-Yamada et al., 2001; Yamada et al., 2002). Systemic administration of bombesin or GRP dose-dependently affects memory consolidation in mice (Flood and Morley, 1988) and rats (Rashidy-Pour and Razvani, 1998). Posttraining administration of GRP also attenuates the memory impairments induced by scopolamine or hypoxia in mice (Santo-Yamada et al., 2001). In addition, the findings that a bombesin-induced intracellular Ca^{2+} release

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mechanism is altered in fibroblasts from patients with Alzheimer disease (Ito et al., 1994) and that bombesin levels are decreased in patients with Parkinson's disease (Bissette et al., 1985) and schizophrenia (Gerner et al., 1985; Olincy et al., 1999) have raised the possibility that dysfunctions in a bombesin/GRP-dependent system play a role in neurodegenerative and neuropsychiatric disorders. Because RC-3095 is a potential clinically useful anticancer drug that acts by blocking bombesin/GRP receptors, and bombesin-like peptides are involved in regulating behavioral and cognitive processes and might play a role in the cognitive decline associated with neurodegenerative disorders, it is important to evaluate the effects of RC-3095 on brain function, including learning and memory processes. We recently reported that RC-3095 impairs aversive memory consolidation when infused into the rat dorsal hippocampus after training (Roesler et al., 2003). In the present study, we investigated the effects of a systemic, acute administration of RC-3095 on two different rodent models of memory, step-down inhibitory avoidance and novel object recognition task in rats.

2. Materials and methods

2.1. Animals

Ninety-two adult female Wistar rats (180–325 g) were obtained from the State Foundation for Health Science Research (FEPPS/LACEN-RS, Porto Alegre, Brazil). Animals were housed five to a cage with food and water available ad libitum, and were maintained on a 12-h light/dark cycle (lights on at 07:00 h). All behavioral procedures were conducted between 10:00 and 16:00 h. All experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

2.2. Drugs and pharmacological procedures

Thirty minutes prior to the behavioral procedures, animals were given an intraperitoneal (i.p.) injection of saline or RC-3095 (0.2, 1.0 or 5.0 mg/kg) (Zentaris AG, Frankfurt, Germany) dissolved in saline, in a volume of 1.0 ml/kg body weight. All drug solutions were prepared immediately before injections.

2.3. Behavioral procedures

2.3.1. Inhibitory avoidance

Inhibitory avoidance in rodents is a widely used animal model of emotionally influenced learning and memory. The step-down inhibitory avoidance apparatus and procedures were described in previous reports (Roesler et al., 1998, 1999, 2000; Picada et al., 2002). Briefly, the inhibitory avoidance box was a 50 × 25 × 25-cm acrylic box whose floor consisted of parallel stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed on the floor of the box against the left wall. Animals were placed on the platform and their latency to step-down on the grid with all four paws was recorded with an automated device. In training sessions, immediately after stepping down on the grid, the animals were given a 0.6 mA, 1.0 s footshock. In two retention test sessions carried out 1.5 (short-term retention) and 24 h (long-term retention) after training, no footshock was given and the step-down latency (maximum 180 s) was used as a measure of retention.

2.3.2. Footshock reactivity

A control experiment evaluating the animals' reactivity to the footshock was carried out in the same apparatus used for inhibitory avoidance, as described in previous reports (Roesler et al., 1999, 2000; Picada et al., 2002). A modified version of the "up-and-down" method (Crocker and Russell, 1984) was used to determine the nociceptive thresholds. The platform was removed and each animal was placed on the grid and allowed a 1-min habituation period prior to the start of a series of footshocks (0.5 s) delivered at 10-s intervals. Shock intensities ranged from 0.1 to 0.8 mA in 0.1-mA increments. The adjustments in shock intensity were made in accordance to each animal's response. Shock intensity was

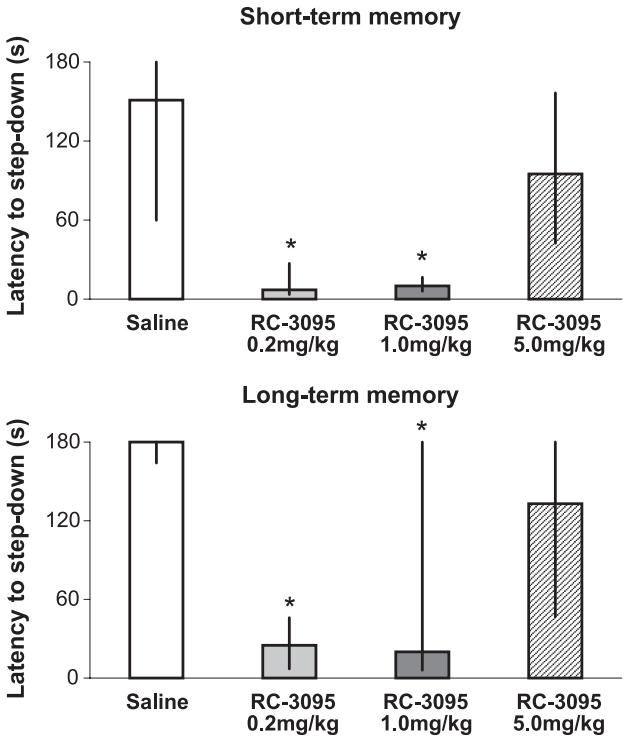

Fig. 1. Effect of pretraining administration of RC-3095 (0.2, 1.0 or 5.0 mg/kg) on short- (1.5 h after training) and long-term (24 h after training) retention of inhibitory avoidance. Animals were given an i.p. injection of saline or RC-3095 30 min prior to training. Data are median (interquartile ranges) retention test latencies (s). $N = 9–11$ animals per group; $^*P < 0.01$ compared to the saline-treated group.

raised by 1 unit when no response occurred and lowered by 1 unit when a response was made. A "flinch" response was defined as withdrawal of one paw from the grid floor and a "jump" response was defined as a rapid withdrawal of three or four paws. Two measurements of the "flinch" threshold were made and then two measures of the "jump" threshold were made. For each animal, the mean of the two scores for the flinch and jump thresholds was considered.

2.3.3. Novel object recognition

The object recognition task in rodents is a nonspatial, nonaversive memory test which has been shown to be a very useful experimental tool for assessing changes in neural function induced by drugs (Dodart et al., 1997; Puma et al., 1999) or genetic manipulations (Tang et al., 1999; Rampon et al., 2000). The task took place in a 40×50-cm^2 open field surrounded by 50-cm high walls, made of brown plywood with a frontal glass wall. All animals were given a habituation session where they were left to freely explore the open field for 5 min. No objects were placed in the box during the habituation trial. Twenty-four hours after habituation, a training trial was conducted by placing individual rats for 5 min into the same open field, in which two identical objects (objects A_1 and A_2; Duplo Lego toys) were positioned in two adjacent corners, 10 cm from the walls. In a short-term memory test given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. The novel object was placed in 50% trials in the right side and 50% trials in the left side of the box. The percentage of the total exploration time that the animal spent investigating the novel object was the measure of recognition memory. Between trials, the objects were washed with 10% ethanol solution. In a long-term memory test given 24 h after training, the same rats explored the field for 5 min in the presence of familiar object A and a novel object C. Recognition memory was evaluated as for the short-term memory test. Exploration was defined as sniffing or touching the object with the nose and/or forepaws.

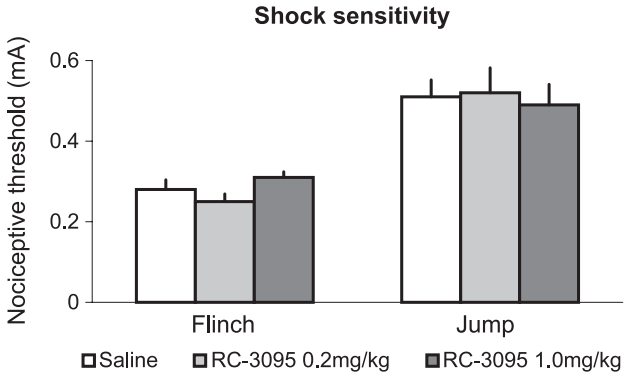

Fig. 2. Effect of RC-3095 (0.2 or 1.0 mg/kg) on reactivity to the footshock. Animals were given an i.p. injection of saline or RC-3095 30 min prior to testing nociceptive thresholds. Data are means ± S.E. nociceptive thresholds (mA). $N = 7$ animals per group. There were no significant differences between groups ($P = 0.30$ for flinch and $P = 0.95$ for jump thresholds).

Table 1
Total amount of time (s) spent exploring both objects during object recognition training in rats given an i.p. injection of saline or RC-3095 (0.2 or 1.0 mg/kg) 30 min before training

Group	*N*	Mean ± S.E. time (s)
Saline	9	25.99 ± 3.37
RC-3095 0.2 mg/kg	10	26.04 ± 3.13
RC-3095 1.0 mg/kg	10	31.02 ± 5.30

Data are expressed as mean ± S.E.; there was no significant difference among groups ($P = 0.61$).

2.4. Statistics

Data for inhibitory avoidance retention and exploratory preferences in the recognition memory task are expressed as median (interquartile ranges). Comparisons among groups were performed using a Kruskal–Wallis analysis of variance followed by Mann–Whitney *U*-tests when necessary. Exploratory preferences within individual groups were analyzed with Wilcoxon tests. Nociceptive thresholds in the footshock reactivity test and total exploration time during object recognition training are expressed as mean ± S.E., and comparison among groups was done using an analysis of variance (ANOVA). In all comparisons, $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Effects of pretraining administration of RC-3095 on short- and long-term retention of inhibitory avoidance

There were no significant differences among groups in training performance ($P = 0.25$; overall mean ± S.E. training latency (s) = 12.09 ± 1.61). Fig. 1 shows the short-term (1.5 h) and long-term (24 h) retention of inhibitory avoidance in rats given an i.p. injection of saline or RC-3095 (0.2, 1.0 or 5.0 mg/kg) 30 min prior to training. RC-3095 at the doses of 0.2 or 1.0 mg/kg, but not at the dose of 5.0 mg/kg, impaired both short- (Fig. 1A) and long-term (Fig. 1B) retention test performance. When retrained and retested drug-free 1 week after the first training, animals previously treated with 0.2 or 1.0 mg/kg RC-3095 and tested for 24-h retention showed normal inhibitory avoidance learning ability (data not shown), indicating that the impairing effects of RC-3095 could not be attributed to an irreversible impairing effect or permanent neuronal damage.

3.2. Lack of effect of RC-3095 on reactivity to the footshock

In order to verify whether the impairing effects of RC-3095 on retention of inhibitory avoidance could be due to

a reduction in nociception, we evaluated footshock sensitivity in animals treated with 0.2 or 1.0 mg/kg RC-3095. Reactivity to the footshock assessed by flinch and jump thresholds was not affected by RC-3095 (Fig. 2), indicating that the memory-impairing effects of pretraining injections of the drug were not due to reduced nociceptive response.

3.3. Lack of effect of RC-3095 on object recognition memory

In the object recognition task, there was no significant difference among groups in the total time exploring both identical objects during training (Table 1), indicating that the pretraining injection of RC-3095 had no effect on exploratory activity or motivation. Results for recognition memory retention are shown in Fig. 3. There were no significant differences among groups in the time exploring any of the two identical objects during training (Fig. 3A) or in time exploring the novel object during either the short- (Fig. 3B) or the long-term memory (Fig. 3C) test. Animals in all three groups showed significant preference towards the novel object during both the short- and long-term memory tests (Wilcoxon tests, $P<0.05$ in all groups). The results show that acute pretraining systemic administration of RC-3095 did not affect novel object recognition memory.

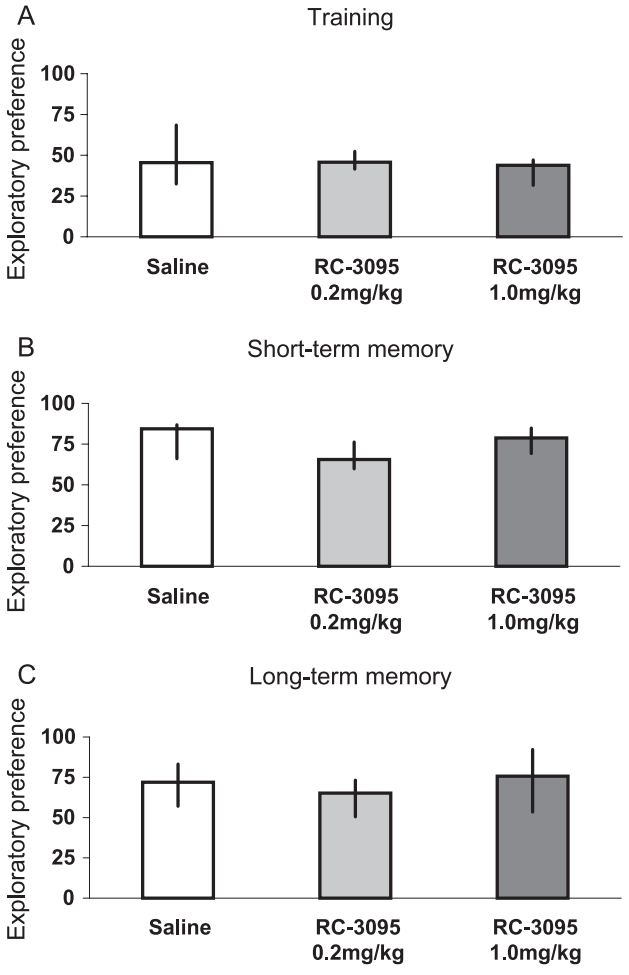

Fig. 3. Effect of 30 min pretraining i.p. injection of RC-3095 (0.2 or 1.0 mg/kg) on memory of a novel object recognition task in rats. Results are shown as median (interquartile ranges). (A) Percentage of time exploring any of the two identical objects during training. (B) Exploratory preference (percentage of time exploring the novel object) in a short-term memory test carried out 1.5 h after training. (C) Exploratory preference (percentage of time exploring the novel object) in a long-term memory test carried out 24 h after training. $N=9-10$ animals per group. All three groups showed significant exploratory preference towards the novel object in both short- and long-term memory trials ($P<0.05$ in all groups). There were no significant differences among groups ($Ps=0.68$ in training, 0.26 in the short-term memory trial and 0.63 in the long-term memory trial).

4. Discussion

The evidence that bombesin-like peptides act as growth factors in the progression of several types of human cancer (Bologna et al., 1989; Moody and Cuttitta, 1993; Siegfried et al., 1994; Wang et al., 1996; Chatzistamou et al., 2001) has led to the development of bombesin/GRP receptor antagonists as potential antitumor agents. RC-3095 is a bombesin receptor antagonist shown to be effective in inhibiting tumor growth in several experimental models (Qin et al., 1994; Szepeshazi et al., 1997; Chatzistamou et al., 2001). Because bombesin-like peptides act as neuropeptides that regulate several aspects of neural function and behavior (Flood and Morley, 1988; McCoy and Avery, 1990; Morley et al., 1992; Williams and McGaugh, 1994; Wada et al., 1998; Santo-Yamada et al., 2001; Yamada et al., 2002) and might as well participate in processes underlying neurodegenerative and neuropsychiatric disorders (Bissette et al., 1985; Gerner et al., 1985; Ito et al., 1994; Olincy et al., 1999), it is important to evaluate the effects of drugs that act by blocking the bombesin/GRP system, such as RC-3095, on brain function. Although a number of recent studies investigate the antitumor activity of RC-3095, to our knowledge, there are no previous reports on the effects peripheral administration of this drug on cognitive processes.

The present study shows that RC-3095 specifically impaired aversive memory formation, without affecting recognition memory, in rats. Since RC-3095 did not affect training performance, exploratory activity, motivation or footshock reactivity, it is unlikely that the memory-impairing effects observed were due to disturbances of sensorimotor function. Because animals were given pretraining injections only, both acquisition and early consolidation processes must have been affected by RC-3095. We recently observed that RC-3095 impairs inhibitory avoidance memory when given into the dorsal hippocampus early after training (Roesler et al., 2003), suggesting that the impairing effect is mediated by an interference on consolidation processes. Future experiments should further evaluate the effects of posttraining and pretest injections of RC-3095 in

order to investigate the specific role of bombesin receptors in memory consolidation and retrieval.

It is interesting to note that RC-3095 impaired avoidance memory at the two lower doses used, while it had no effect at the higher dose. Several studies evaluating the effects of injections of both memory-enhancing (McGaugh, 1989) and memory-impairing (Packard and Teather, 1997) drugs on memory show that many posttraining treatments produce an inverted-U dose–response curve where specific doses are optimal whereas both lower and higher doses are ineffective (McGaugh, 1989; Packard and Teather, 1997). This dose–response pattern is also consistent with that of intrahippocampal infusions of RC-3095 (Roesler et al., 2003).

The present finding that peripheral administration of the bombesin/GRP receptor antagonist RC-3095 impairs the formation of memory for inhibitory avoidance in rats is consistent with previous reports that bombesin-like peptides enhance memory in rodents when given systemically (Flood and Morley, 1988). Several brain areas and neuronal signaling pathways might be involved in the impairment of aversive memory induced by peripheral injection of RC-3095. Brain areas importantly involved in the formation of inhibitory avoidance memory, such as the hippocampus and amygdala, contain high densities of bombesin/GRP receptors (Moody et al., 1978; Pert et al., 1980; Wolf and Moody, 1985; Zarbin et al., 1985). In addition, application of bombesin-like peptides to rat hippocampal neurons produces a large membrane depolarization (Lee et al., 1999), and intracellular responses to the activation of bombesin/GRP receptors in several cell types have been shown to involve protein kinase signaling pathways, such as the protein kinase A (PKA), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated protein kinase (ERK) pathways (Hellmich et al., 1999; Kim et al., 2000; Qu et al., 2002) that are also involved in neural plasticity and memory formation in the rat hippocampus (Izquierdo and Medina, 1997; McGaugh, 2000; McGaugh and Izquierdo, 2000). Since infusion of bombesin into the nucleus of the solitary tract enhances consolidation of memory for inhibitory avoidance in rats (Williams and McGaugh, 1994), whereas lidocaine-induced functional inactivation of the nucleus of the solitary tract and amygdala attenuates the bombesin-induced enhancement of retention in rats (Rashidy-Pour and Razvani, 1998), the nucleus of the solitary tract and amygdala are also likely to be involved in the memory modulation by bombesin/GRP receptors, and those brain areas might as well be involved in mediating the memory-impairing effect of RC-3095 reported in the present study. Further studies are required to clarify the brain areas and signaling pathways involved in the RC-3095-induced memory impairment.

It is possible that bombesin-like peptides are also involved in the memory deficits associated with degenerative brain disorders. The finding by Ito et al. (1994) that fibroblasts from patients with Alzheimer's disease show altered bombesin-induced, inositol trisphosphate-dependent Ca^{2+} released has suggested that alterations in a bombesin/GRP receptor-dependent intracellular Ca^{2+} mobilization mechanism might play a role in the cognitive deficits observed in patients with Alzheimer's disease. In addition, schizophrenic patients show reduced urine and cerebrospinal fluid levels of bombesin (Gerner et al., 1985; Olincy et al., 1999), and the concentration of bombesin is significantly decreased in the brain tissue from patients with Parkinson's disease (Bissette et al., 1985). Thus, the investigation of the effects of bombesin/GRP receptor antagonists on animal models of cognitive function and neuropsychiatric disorders might be relevant for understanding the neurodegenerative processes and the proposal of new molecular targets for neuroprotection.

RC-3095 impaired inhibitory avoidance retention but did not affect memory of a novel object recognition task when injected at the same doses shown to impair inhibitory avoidance. Whereas inhibitory avoidance is a type of aversive, context-dependent memory (Roesler et al., 1998, 1999, 2000; see Izquierdo and Medina, 1997; McGaugh, 2000; McGaugh and Izquierdo, 2000 for reviews), object recognition is a nonaversive, nonspatial memory task (Dodart et al., 1997; Puma et al., 1999; Tang et al., 1999). Although formation of memory of both tasks has been shown to share some similar mechanisms (for instance, both tasks are dependent on glutamate receptors in the hippocampus) (Izquierdo and Medina, 1997; Roesler et al., 1998; Rampon et al., 2000), our findings suggest that these two types of memory are differentially regulated by the bombesin/GRP system. It is possible that bombesin-like peptides are more importantly involved in modulating emotionally influenced, stressful memory tasks like inhibitory avoidance than nonaversive tasks like recognition memory. This hypothesis would be consistent with evidence from a recent study using bombesin-like peptide receptor knockout mice, which has shown that bombesin modulates emotional and anxiety-related behavior (Yamada et al., 2002).

In summary, the present study shows that the antitumor drug RC-3095, a bombesin/GRP receptor antagonist, impairs aversive memory without affecting recognition memory in rats when given peripherally prior to training. Given that bombesin/GRP receptor antagonists such as RC-3095 are potential clinically effective antitumor agents that affect the central nervous system, the present might be considered a preclinical indication that these drugs might induce cognitive impairments in patients. Future studies should further evaluate the effects of these drugs on normal neural function as well as in neurological and psychiatric disorders in humans.

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Note added in proof

After the first version of the present article was submitted for publication, a study reporting impairing effects of systemic administration of bombesin/GRP receptor antagonists on inhibitory avoidance memory in mice was published by Santo-Yamada et al. (Neurosci Lett 2003, 340, 65–68). The findings reported by Santo-Yamada et al. are consistent with the results of the present study and support our view that bombesin/GRP receptors are required for formation of aversive memory. In addition, we recently found evidence (Roesler et al., Eur J Neurosci, in press) that the basolateral amygdala mediates the imparing effects of RC3095 on memory for inhibitory avoidance.

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