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Apomorphine Enhances Conditioned Responses Induced by Aversive Stimulation of the Inferior Colliculus

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Consistent evidence has shown that learning may be produced in paradigms using electrical stimulation of the inferior colliculus (IC) as unconditioned stimulus (UCS). Recent reports have also demonstrated that aversive stimulation of the IC, at the escape threshold, enhances dopamine (DA) release in the prefrontal cortex. The purpose of the present study was to determine whether dopaminergic mechanisms are involved in the Pavlovian conditioning and latent inhibition using IC stimulation as UCS and light as conditioned stimulus (CS). Rats were placed inside a shuttle box and subjected to a two-way avoidance paradigm. IC aversive electrical stimulation was used as UCS and shuttle box illumination as CS. The rats quickly learned to avoid or interrupt the IC stimulation. Apomorphine injections produced a dose-dependent increase in the number of avoidance responses. On the other hand, chlorpromazine administration promoted a dose-dependent reduction of the avoidance responses. Previous injections of chlorpromazine inhibited the effects of apomorphine. Also, previous exposure to unreinforced light weakened the strength of the conditioning. Apomorphine blocked this latent inhibition effect, which was antagonized by previous injections of chlorpromazine. These findings bring evidence for the involvement of DA in the setting up of adaptive responses to aversive states generated at the IC level, which may underlie stressful situations present in anxiety.

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INTRODUCTION

The search for the subcortical organization of fear processing dates back to the beginning of the last century with seminal work by Bard (1929) indicating the hypothalamus as an important center of integration of defensive reactions. Two decades later, low mesencephalic levels were also implicated in these processes in a report with detailed observations of the behavior of chronically decerebrate cats, in which the full capabilities of hypothalamic animals were compared with those of mesencephalic and bulbospinal preparations (Bard and Match, 1951). An intriguing aspect on these studies was that the stimulus used on all occasions was a sound. The inferior colliculus (IC) is a midbrain tectum structure primarily involved in auditory information processing; it also integrates sensory information of aversive nature. Indeed, electrical or chemical stimulation of this structure induces responses such as freezing and flight responses that mimic fearful behavior elicited by environmental challenges (Schmitt *et al*, 1986; Brandão *et al*, 1988, 1994, 1999; Cardoso *et al*, 1994; Troncoso *et al*, 1998; Castilho *et al*, 1999). Several studies have suggested modulatory roles for GABA, serotonin, opioids, excitatory amino acids and neuropeptides at the so-called brain aversion system, which includes the dorsal periaqueductal gray and IC (Brandão *et al*, 1982, 1994, 1999). The IC is connected to cortical areas (Adams, 1979; Hoffman and Ison, 1980; Meininger *et al*, 1986; Fuster, 1989; Hoffman Brodal, 1992). One of these pathways is provided by projections from the central nucleus of the IC to the prefrontal cortex through the medial geniculate nucleus, amygdala and dorsomedial thalamus (Fuster, 1989; Brodal, 1992). It has been shown that this circuit is concerned with the processing of auditory information of an aversive nature which triggers fear-like behaviors (Maisonnette *et al*, Hoffman 1996; Brandão *et al*, 1999).

It has been found that local application of the excitant amino acids, glutamate and NMDA, in the central nucleus of the IC resulted in increased acoustically evoked and spontaneous firing of most neurons of this region (Li *et al*, 1998; Faingold *et al*, 1989). Recently, we have shown that like fear-evoking stimuli, apomorphine increases the magnitude of the evoked potential to loud sounds directly recorded from the central nucleus of the IC (Brandão *et al*, 2001; Sandner *et al*, 2002). Interestingly enough, aversive stimulation of this midbrain structure, at the escape threshold, enhances dopamine (DA) release in the prefrontal cortex (Cuadra *et al*, 2000). We believe that studies

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directed to the research of DA-mediated mechanisms of these processes have considerable relevance in the light of the changes at DA transmission that occur following external acute stressors (Fadda *et al*, 1978; Anisman *et al*, 1991; Biggio *et al*, 1990; Feenstra *et al*, 1995; Feenstra and Botterblom, 1996) and of the current knowledge that cortical DA projections are also activated by a wide variety of aversive stimulations (Thierry *et al*, 1976; Fadda *et al*, 1978; Deutch *et al*, 1985; Abercrombie *et al*, 1989; Feenstra and Botterblom, 1996; Goldstein *et al*, 1996).

Latent inhibition is the interference with associative learning by prior unreinforced exposures to a conditioned stimulus (Makintosh 1975; Lubow *et al*, 1982). Latent inhibition has been considered as a model for information filtering processesand demonstrates attentional processes in rats that are responsive to drug treatment in a fashion that parallels drug responses seen in humans with schizophrenia (Makintosh, 1975; Lubow *et al*, 1982; Dunn *et al*, 1993; Sandner *et al*, 2002). We have already shown that stimulation of neural circuits in the central nucleus of the IC supports Pavlovian conditioning and that latent inhibition may be demonstrated using stimulation of these neural circuits in the IC as negative reinforcement (Brandão *et al*, 1997; Troncoso *et al*, 1998; Castilho and Brandão, 2001).

As the IC—a primary acoustic structure of the brainstem—seems to have a functional link with other higher brain structures through dopaminergic mechanisms, the purpose of the present study was to detect dopaminergic activity in the IC and to determine whether DA mechanisms are involved in the Pavlovian conditioning and latent inhibition using IC stimulation as unconditioned stimulus (UCS). To this end, we used a two-way active avoidance procedure in which light was used as the warning signal (conditioned stimulus (CS)) for an electrical stimulation train applied to the IC. Electrical stimulation of the IC was then the UCS paired with the box illumination as a CS. For the latent inhibition procedure, unreinforced light stimuli were presented before the sessions. We examined the effects of systemic injections of the classical DA agonist apomorphine and the classical DA postsynaptic receptor antagonist chlorpromazine on these paradigms.

MATERIALS AND METHODS

Animals

A total of 92 male Wistar rats weighing 250–300 g were housed in individual Plexiglas-walled cages under a 12:12 dark/light cycle (lights on at 07:00 am) at $23 \pm 1^{\circ}$C and given free access to food and water throughout the experiment. The experiments reported in this article were performed in compliance with the recommendations of SBNeC (Brazilian Society of Neuroscience and Behavior), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

Surgery

The animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and fixed in a stereotaxic frame (David Kopf, USA). A brain electrode was implanted in the midbrain, aimed at the IC. The electrode was made of stainless-steel wire, 160 μm in diameter, insulated except at the cross-section of the tip. The upper incisor bar was set at 3.3 mm below the interaural line, so that the skull was horizontal between bregma and lambda. The electrode was introduced vertically using the following coordinates, with the lambda serving as the reference for each plane: postero-anterior, 1.2 mm; medio-lateral, 1.5 mm; dorso-ventral, 4.5 mm (Paxinos and Watson, 1997). The electrode was fixed to the skull by means of acrylic resin and three stainless-steel screws. The electrode wire was connected to a connector so that it could be plugged into an amphenol socket at the end of a flexible electrical cable used for brain stimulation.

Apparatus

One week after surgery, the rats were placed in an open field—a circular enclosure 60 cm in diameter and 50 cm high. The rats were allowed a 15 min period of habituation in the enclosure. The brain was stimulated electrically by means of a sine wave stimulator (Marseillan, 1977). The stimulation current was monitored by measuring the voltage drop across a 1 kΩ resistor with an oscilloscope (Labo, Brazil). Brain stimulation (AC, 60 Hz, 15 s) was presented at 1 min intervals with the current intensity increasing by steps of 1.4 μA (rms) for measurements of the escape threshold. Escape threshold was operationally defined as the lowest current intensity that produced running or jumping in two successive ascending series of electrical stimulation. Animals with an escape threshold above 70 μA (rms) were discarded from the experiment.

The active avoidance cage consisted of a shuttle box comprising two compartments $30 \times 25 \times 25$ cm with a 2.5 cm high barrier between them, and was equipped with 4 photoelectrical cells equally spaced on the back wall. This arrangement allowed one to detect the shuttle locomotion of the rat as well as its gross locomotor activity within each compartment. The grid floor consisted of bars spaced 1.2 cm apart. A 28 V light bulb was centered on the rear wall of each compartment of the chamber, 12 cm from the floor. The light was turned on and off noiselessly. The IC stimulation was delivered by a protected wire lead that entered the conditioning chamber through a 2 cm hole located in the top wall of the chamber. The rat was placed in the shuttle box and had its brain electrode connected to a flexible wire cable, allowing ample movement inside the box. The cable, in turn, was connected to the stimulator by means of a mercury swivel mounted on the top of the experimental chamber. The brain stimulation was applied at a current intensity 5% below the escape threshold previously determined in the open field. The adequacy of this current intensity level for the escape response was verified on the basis of a previous study from this laboratory (Melo *et al*, 1992; Brandão *et al*, 1997; Troncoso *et al*, 1998).

Procedure

After the determination of the aversive threshold, the animals were placed inside the shuttle box (10 lx at the floor level) and left for 15 min for habituation to the experimental context before the beginning of the session. The animals

were randomly assigned to five groups, which received one of the following treatments: (a) 0.5 mg/kg of apomorphine, $n=9$; (b) 2.0 mg/kg of apomorphine, $n=9$; (c) 1.0 mg/kg of chlorpromazine, $n=8$; (d) 2.0 mg/kg of chlorpromazine, $n=8$. The fifth group served as the control group and received i.p. injections of the vehicle ($n=8$). All injections were given 30 min before the sessions. After these groups were tested, an additional group of animals ($n=8$) was included in which 0.5 mg/kg of apomorphine was challenged with 2.0 mg/kg of chlorpromazine. The animals were submitted to a session that consisted of 50 trials. Two successive trials were separated by a random interval from 10 to 50 s. Each animal was submitted to only one session. During each conditioning trial, the shuttle box was illuminated for 20 s (100 lx at the floor level) followed by an electrical stimulation (AC, 60 Hz, 10 s) applied through the implanted electrode. Whenever a rat passed from one compartment to the other during the illumination, it avoided the brain stimulation (avoidance responses = latency below 20 s); if it changed compartments during electrical stimulation of the brain, then the stimulation was automatically terminated (escape responses = latency between 20 and 30 s). The latencies and the number of avoidance and escape responses were individually recorded as well as the intertrial locomotor activity.

For the latent inhibition experiments, the two-way avoidance session also consisted of 50 trials. Each animal received 50 pre-exposures to the CS (light) in the shuttle box before the session, instead of experiencing the period of habituation of the conditioning experiment. The animals were divided into four groups that were allocated to the following drug treatments: (a) vehicle (saline, control group), $n=8$; (b) 0.5 mg/kg of apomorphine, $n=8$; (c) 2.0 mg/kg of chlorpromazine, $n=8$; (d) chlorpromazine (2.0 mg/kg)+apomorphine (0.5 mg/kg), $n=8$. In these experiments drugs were injected immediately before the sessions.

The presentation and termination of the conditioned and unconditioned stimuli, along with all data collection, were controlled by a PC computer connected through an interface to the experimental chamber.

Drugs

Apomorphine hydrochloride 0.5. and 2.0 mg/kg (Sigma, USA) and chlorpromazine hydrochloride 1.0 and 2.0 mg/kg (Sigma, USA) were each dissolved in physiological saline (0.9%) shortly before use. Doses and waiting time for drug effects were chosen on the basis of the peak of effects of these DA agents observed in a preliminary study of this laboratory (Furlan and Brandão, 2001). The doses of the drugs were administered in a constant volume of 1 ml/kg, i.p.

Histology

Upon completion of the experiments, the animals were deeply anesthetized with sodium pentobarbital and perfused intracardially with saline followed by formalin solution (10%). Three days later the brains were removed and frozen. Serial 50 μm brain sections were cut using a microtome and stained with Evans blue (2%) in order to localize the positions of the electrode tips according to the Paxinos and Watson atlas (1997).

Monoamine Assays

The animals ($n=10$) were killed by decapitation, the brain was quickly removed and the central nucleus of the IC of both sides was punched out, weighed and kept at -80° until DA and 3,4-dihydroxyphenylacetic acid (DOPAC) were measured by high-pressure liquid chromatography (HPLC). The HPLC system was equipped with a reverse-phase column (Shim Pack CLC-OSD (M) 25 cm, 5 μm and 100 Å pore diameter particle size; Shimadzu, Kyoto, Japan), coupled with electrochemical detection. The samples were homogenized in percloric acid 0.2 M, centrifuged at 15 000 rpm during 20 min, at -6°C, and 20 μl were injected into the HPLC-EC system. The HPLC system consisted of a Shimadzu chromatograph LC-10 AD, with a communication bus module CBM-10A, an on-line degassing unit DGU-14A, an L-ECD – 6A electrochemical detector with a glass–carbon electrode and a pump LC-10 AD. The potential was set at 850 mV (*vs* a Ag/AgCl reference electrode). The mobile phase containing 149 mM citric acid, 10 mM of NaCl, 800 ml of distilled water, 1-octane sulfonic acid, 48 ml of acetylnitrile and 28 ml of tetrahydrofurane, (pH 3.0) was filtered and pumped through the system at a flow rate of 1.0 ml/min. Quantification of all substances was made by comparing the peak area to a standard.

Analysis of Results

Data are reported as mean $\pm$ SEM. Latencies for avoidance and frequency of avoidance responses obtained in the conditioning experiments were subjected to a two-way analysis of variance (ANOVA) with repeated measures using the drug condition as a between factor and blocks as the repeated measure factor. For the latent inhibition experiment, one-way ANOVA was used to analyze both frequencies and latencies of responses in the entire session. *Post hoc* differences between group means were tested with the Newman–Keuls test. A P lower than 0.05 was considered significant.

The DOPAC/DA ratio was used to calculate the dopaminergic activity in the IC. Comparison of the data of DA, DOPAC and dopaminergic activities was done by Student's t-test. A P value of $<5\%$ was considered significant.

RESULTS

The tips of the electrodes were situated inside the central nucleus of the IC as shown in Figure 1. Gradual increases in the current intensity induced alertness, freezing and escape reactions expressed by running and/or jumping accompanied by piloerection, defecation and micturition. No audible vocalizations could be detected during the stimulation of the IC of the animals used in this study. The intensity of the electric current applied to the IC of the animals to induce escape responses was 54.12 ± 6.25 μA (rms).

Figure 2a presents the mean frequency of avoidance responses of the six groups across the sessions blocks. Significant differences emerged between treatments: drug effects $F(5, 176) = 10.28$, $P<0.001$. Overall, the number of

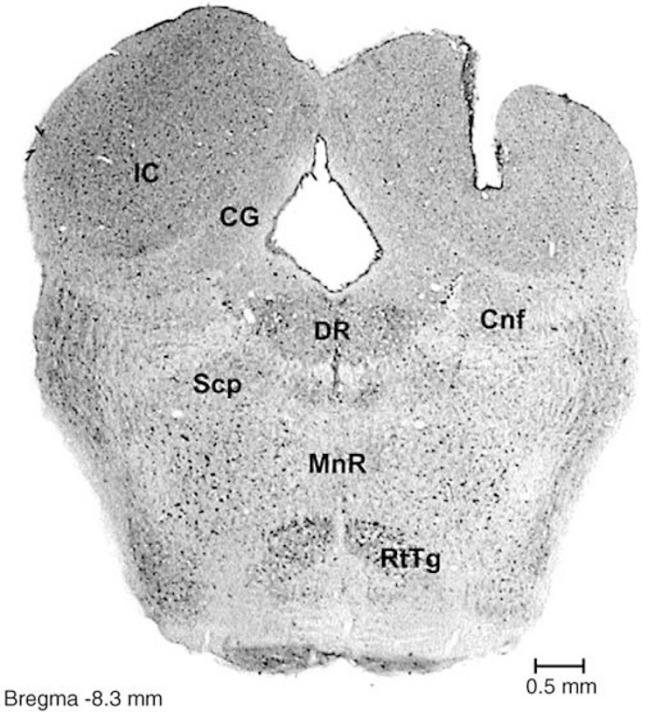

Figure 1 Photomicrograph showing a typical example of an electrode implanted in the central nucleus of the IC. IC: inferior colliculus; DR: dorsal raphe nucleus; SCP: superior cerebellar peduncle; RtTg: reticulopontine tegmental nucleus; Cnf: cuneiform nucleus; CG: central gray; MnR: median raphe nucleus. Bar = 500 μm.

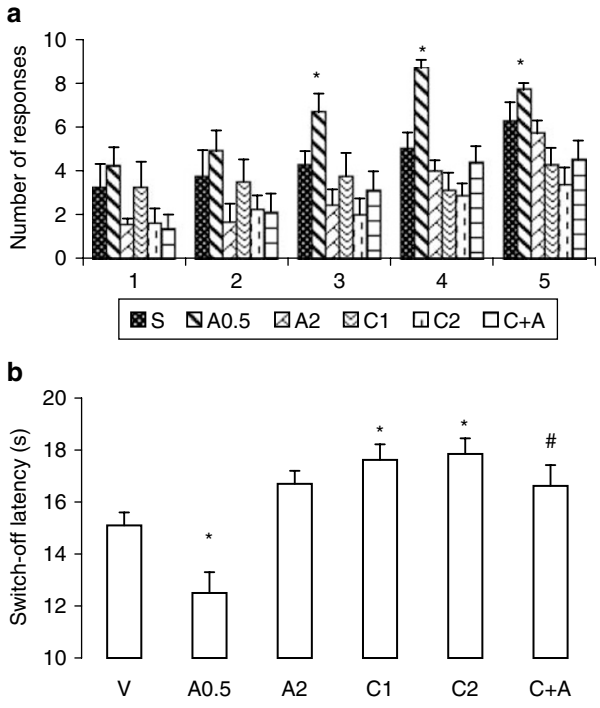

Figure 2 (a) Mean (± SEM) of avoidance responses across blocks of 10 trials during sessions with six groups of rats injected with saline, 0.5 and 2.0 mg/kg of apomorphine, 1.0 and 2.0 mg/kg of chlorpromazine and chlorpromazine (2.0 mg/kg)+apomorphine (0.5 mg/kg), and submitted to 50 trials of conditioning with pairing electrical stimulation of the IC with neutral conditioned stimulus (box illumination). (b) Latencies of avoidance responses in the same experimental situation. Drugs were injected 30 min before the sessions. *$P<0.05$ in relation to the control group injected with saline and #$P<0.05$ in relation to the group injected with apomorphine 0.5 mg/kg, Newman–Keuls comparisons. S, saline; A, apomorphine; C, chlorpromazine. $N=8$ for saline, 1 and 2 mg/kg of chlorpromazine, $N=9$ for apomorphine 0.5 and 2.0 mg/kg and $N=8$ for the chlorpromazine+ apomorphine group.

avoidance responses significantly increased across blocks of 10 trials: block effect, $F(4, 176)=13.34$, $P<0.001$. These changes across blocks, however, did not vary as a function of drug treatment: treatment × blocks interaction, $F(20, 176)=0.27$, $P>0.05$. Inspection of Figure 2a indicates that control rats showed increased avoidance responses across blocks. Figure 2b presents the latencies for avoidance responses of the six groups in the whole session. As can be seen, these measures were also significantly changed by treatments, $F(5, 44)=12.17$, $P<0.01$. *Post hoc* the Newman–Keuls method showed that the significant effects were caused by differences between the treatment with apomorphine (0.5 mg/kg) and chlorpromazine (1 and 2 mg/kg) in relation to the control group. Here also, previous injections of chlorpromazine 2 mg/kg inhibited the effects of apomorphine 0.5 mg/kg. The enhancement of the acquisition of the conditioned responses caused by apomorphine 0.5 mg/kg was clearly reduced by previous injection of chlorpromazine. The analyses are consistent with the assertion that dopaminergic agonists strengthen the acquisition of conditioned responses and that classical antipsychotic agents as chlorpromazine reduce the avoidance responses to aversive stimulation. These data are shown in Figure 2b.

Analysis of the intertrial locomotor activity (crossings) during the conditioning sessions did not reveal any particular drug effects on the motor performance of the animals ($F5, 44=1.63$, $P>005$). In general, escape responses always occurred when the animals did not respond over the 20-s period of light CS. The latencies of escape responses did not change across treatment groups ($F(5, 44)=1.84$, $P>0.05$).

ANOVA performed on the data obtained with latent inhibition experiments revealed significant effects of drug injections upon the number ($F(4, 39)=6.92$, $P<0.001$) and latencies ($F(4, 39)=6.07$, $P<0.001$) of avoidance responses. Newman-Keuls test showed that the pre-exposure group injected with saline showed a significant reduction in the avoidance behavior, while apomorphine injections (0.5 mg/kg) caused a significant increase in this behavior. The effects of apomorphine were clearly inhibited by a previous injection of chlorpromazine 1 mg/kg. The effects of chlorpromazine on its own on these measures were not different from the saline pre-exposed group. These data are depicted in Figure 3.

Table 1 shows the data of the neurochemical assays comparing the DA activity in the left and right IC. There were no differences ($df=18$) in the levels of DA ($t=0.84$, $P>0.05$), DOPAC ($t=0.99$, $P>0.05$) and the DOPAC/DA ratio ($t=1.54$, $P>0.05$) in both central nuclei of the IC.

DISCUSSION

All stimulated sites producing defensive behavior in the present study were situated in the ventral aspect of the

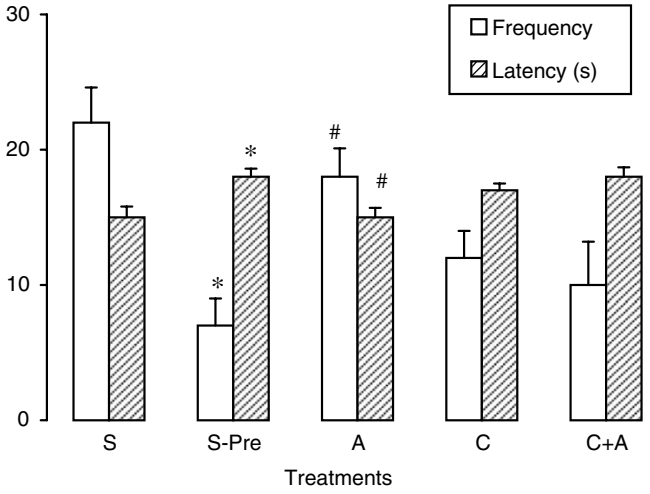

Figure 3 Effects of apomorphine (0.5 mg/kg, i.p.) on the latent inhibition caused by 50 light pre-exposures before the session of two-way avoidance (mean ± SEM). Each animal was injected with saline or apomorphine immediately before the sessions in the pre-exposed groups. Apomorphine was injected soon after chlorpromazine in the group C+A. Open columns: number of avoidance responses. Hatched columns: latencies for avoidance responses. * different from the non pre-exposed group injected with saline and # different from the pre-exposed group treated with saline (Newman–Keuls test, $P<0.05$). S, saline; A, apomorphine; C, chlorpromazine (2.0 mg/kg). $N=8$ for each group.

Table 1 Levels in ng/g of Brain Tissue and Metabolite-to-Transmitter Ratios (means ± SEM) in the Left and Right IC[1]

	DA	DOPAC	Ratio
IC left	79.02 ± 6.54	50.25 ± 5.98	0.63 ± 0.08
IC right	84.30 ± 5.32	44.30 ± 4.74	0.53 ± 0.07

No differences between the left and right IC could be detected in all cases. $P>0.05$, paired *t*-test. $N=10$. [1]DA, dopamine, DOPAC, 3,4-dihydroxyphenylacetic acid.

central nucleus of the IC, a region traditionally known to process high-pitched sounds (Rose *et al*, 1963; Merzenich and Reid, 1974). The present results show that rats quickly learn to make a shuttling response in order to avoid or escape from the electrical stimulation of the IC. This represents a consistent demonstration of learning using aversive states induced by IC stimulation as UCS and light stimulation as CS. The rats increased their rate of responding in the presence of the CS (cage illumination) over number of trials. The pattern of results obtained in the present experiments parallels those from typical avoidance–escape procedures, which utilize electric shock as the UCS, for example, an auditory stimulus paired with foot shock elicits classical conditioned responses (LeDoux *et al*, 1986, 1990). From the anatomo-functional viewpoint, the IC may be associated with the processing of aversive conditioned stimuli and, for extension, with conditioned fear (Brandão *et al*, 2001; Castilho and Brandão, 2001). Therefore, the present results are in line with recent evidence obtained in this laboratory that has shown that rats learn to avoid the IC stimulation when the warning stimulus is a light and not a tone (Troncoso *et al*, 1998).

In accordance with several studies (Brandão *et al*, 1994, 1999), the present results show that escape behavior is normally obtained with gradual increases in the intensity of electrical stimulation of the IC. The fear-like nature of this stimulation has been previously shown in this laboratory, as rats submitted to avoidance paradigms avoid and quickly learn to switch off such stimulation in the IC (Melo *et al*, 1992; Troncoso *et al*, 1998). Nevertheless, we cannot discard the current spreading to neighboring structures such as dorsal periaqueductal gray or cuneiform nucleus, other structures whose stimulation is known to cause defensive behaviors (Amano *et al*, 1978; Nashold *et al*, 1969; Bandler *et al*, 1985; Redgrave *et al*, 1988; Dean *et al*, 1988; Mitchell *et al*, 1988; Graeff, 1994). We predict the existence of neural substrates for defensive behavior in the IC, not only because of the aversive properties of its electrical stimulation but mainly because chemical stimulation with bicuculline or excitatory amino acids such as NMDA and glutamate causes similar aversive responses (Brandão *et al*, 1999, Pandossio *et al*, 1999). Moreover, systemic or local injections of GABA agonists or benzodiazepines into this midbrain structure promptly reduce the aversive consequences of their electrical stimulation (Melo *et al*, 1992; Pandossio and Brandão, 1999). As GABA receptor antagonists mimic the effects of the electrical stimulation, it has been suggested that the neural substrate of aversion in this midbrain region be under GABAergic tonic inhibitory modulation (Melo *et al*, 1992; Brandão *et al*, 1988, 1993, 1994, 1997). Local injections of serotonergic agents known for their antiaversive action when injected in other structures of the brain aversion system also decrease the aversiveness of the exposure of rats to the elevated plus-maze test (Melo and Brandao, 1995).

Based on evidence from this and other laboratories, we have proposed that the IC contributes to some basic link between auditory inputs of aversive nature, and the generation and elaboration of defensive behavior in response to threat or danger (see Brandão *et al* (1999) for a review on this topic). Furthermore, we have shown that electrical stimulation of the IC of rats in intensity sufficient to induce escape behavior enhances subsequent fear-like behaviors when behaviorally tested in the elevated plus-maze test (Pandossio *et al*, 2000).

Apomorphine 0.5 mg/kg clearly enhanced the aversive effects caused by electrical stimulation of the IC, while 2.0 mg/kg did not produce changes in the aversiveness of this stimulation. Activation of dopaminergic receptors by this dopaminergic agent seems to account for this behavioral sensitization as chlorpromazine 2.0 mg/kg, a dose that did not cause apparent change in the animal motor activity, significantly counteracted the effects of apomorphine. Besides, the observed effects of these dopaminergic drugs cannot be attributed to nonspecific effects, as they did not affect the intertrial motor activity or the escape latencies of the animals in the two-way avoidance procedure. The differential effects of the two doses of apomorphine in the present study is consonant with several other studies that report either an increase or a decrease in receptor sensitivity with low and high doses of apomorphine, respectively. To determine a reason for these differential effects of systemically administered apomorphine has been a complicated matter because the drug acts

on several DA pathways, as it acts differentially at different DA subtypes. For example, apomorphine has been reported to be a full agonist at D2 receptor sites (Creese *et al*, 1983). Moreover, although D2 receptors are found both pre- and postsynaptically, it has been reported that the presynaptic sites are between 6 and 10 times more sensitive to apomorphine than the postsynaptic sites (Skirboll *et al*, 1979). As expected, chlorpromazine produced a dose-dependent reduction in animal reactivity to the aversive stimulation of the IC. A local action of apomorphine in the IC cannot be discarded, as appreciable dopaminergic activity exists therein as shown by the present study and also because this drug enhances the auditory-evoked potentials directly recorded from the central nucleus of this midbrain region (Sandner *et al*, 2002). Strong support to our neurochemical data showing significant concentrations of DA in the IC is provided by a recent paper that demonstrate the presence of mRNA for D2 but not D1-receptors in human IC (Hurdy *et al*, 2001). Besides, substantia nigra does not seem necessary for this dopaminergic activity as recent evidence has shown that lesions of this structure, pars compacta included, did not affect the DA levels in the IC (Maisonnette *et al*, 1998). Further studies using systemic and local injections into the IC of selective D1- and D2-receptor antagonists on apomorphine's enhancement of learning will help to elucidate this point.

The association of changes in dopaminergic transmission and threatening challenges has already been demonstrated by numerous reports. In fact, alterations of DA transmission always occur following the exposure to a wide variety of acute stressors (Anisman *et al*, 1991) and cortical DA projections are also activated by diverse types of aversive stimulation (Thierry *et al*, 1976; Fadda *et al*, 1978; Anisman *et al*, 1991; Feenstra *et al*, 1995; Feenstra and Botterblom, 1996; Goldstein *et al*, 1996). Although the precise neural circuit of the DA transmission involved in aversive states remains unclear, pharmacological and neurochemical studies seem to point to DA prefrontal neurons (Espejo and Miñano, 1999; Morrow *et al*, 1999). Support for a functional link between the activation of DA prefrontal neurons and the behavioral responses induced by IC aversive stimulation has been reported recently (Cuadra *et al*, 2000). Although there are substantial cortical inputs to dorsal midbrain regions, it is established that the IC also projects densely to cortical areas, mainly to the temporal lobe (Cooper and Young, 1976; Fadda *et al*, 1978; Adams, 1979; Meininger *et al*, 1986). In fact, the IC is a key pathway for auditory information and disturbances at this level may alter the transmission to cortical centers. An indirect pathway connecting these structures is given by projections from the central nucleus of the IC–medial geniculate nucleus–amygdala–dorsomedial thalamus–prefrontal cortex (Fuster, 1989; Brodal, 1992; Cardoso *et al*, 1994). It has been shown that this alternate circuit is concerned with the processing of auditory information of aversive nature, which triggers fear-like behaviors (LeDoux *et al*, 1990; Maisonnette *et al*, 1996). Support to this assumption comes from a recent paper demonstrating that microinjections of nefazodone, a serotonin antagonist, into the basolateral nucleus of amygdala reduce the aversive reactions induced by NMDA microinjections into the IC (Maisonnette *et al*, 2000).

The present results are in line with recent evidence obtained in this laboratory showing that latent inhibition, considered to be an animal model of schizophrenia, may be demonstrated using stimulation of neural circuits in the IC involved in the processing of acoustic information of aversive nature as negative reinforcement (Brandão *et al*, 1997). Also, our data clearly show that apomorphine inhibits the latent inhibition so induced and that these effects were antagonized by previous injections of chlorpromazine. Abnormal cortical areas are known to exist in schizophrenic patients and may account for the abnormal processing of auditory information consisting of auditory hallucinations and a decreased responsiveness to sounds (David *et al*, 1996). It has been claimed that a global control of the cortical function involved with sensory processing by the mesolimbic system needs to be considered in this field of study (Melo *et al*, 1997). These authors found that bilateral ablation of the auditory cortex enhances latent inhibition using a conditioned suppression response procedure. Taken together, the present results suggest that the IC supports the acquisition of conditioned responses, and that these paradigms may be useful for assessing the neurochemical substrates underlying the aversive effects of the stimulation of the IC.

The effects of apomorphine on latent inhibition remain the focus of some debate (Campeau and Davis, 1995). The differences reported among different experiments on the sensitivity of latent inhibition to apomorphine probably depend on the experimental conditions (Davis *et al*, 1990; Campeau and Davis, 1995) and the rat strain used (Swerdlow *et al*, 2000).

In summary, this work opens a new line of investigation relating neural substrates of aversion and fear within structures of the so-called brain aversion system at the midbrain tectum and DA-mediated mechanisms. Although more investigations are needed to clarify this field, our findings bring new evidence for a possible role of dopaminergic mechanisms in thesetting up of adaptive responses to threatening situations, which activate the neural circuits of the defensive behavior at the IC level.

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