The α_2 Agonist Guanfacine Reinstates Amphetamine-Disrupted Latent Inhibition in Rats

Running Head: GUANFACINE REINSTATES DISRUPTED LI

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Abstract

A functional interaction between an animal and its environment requires appropriate input filtering. Latent inhibition (LI) - the inability to form a new association with a stimulus that the animals previously learned to ignore- is one of the mechanisms by which such filtering is accomplished. While the role of dopamine receptors in LI has been widely investigated, recent evidence suggests that norepinephrine (NE) α_2 receptors might play additional role. To further investigate the role of NE in LI, Sprague-Dawley rats were water-deprived and either preconditioned to ignore a tone, or had no prior exposure to the tone. Subsequently, animals of both groups were conditioned to associate the same tone with a footshock. On the following testing day rats' lick rates before and after a presentation of the same tone without the shock were measured. Preexposed animals demonstrated much lower rates of association than non-preexposed animals. Amphetamine administration (1mg/kg, 30 min pre-testing) abolished the behavioral difference between preexposed and non-preexposed groups. We tested the hypothesis that α_2 adrenoceptors were involved in LI by treating rats with the specific NE α_2 agonist guanfacine (0.3mg/kg, 60 min pre-testing). Guanfacine reversed the disrupting effect of the amphetamine administration reinstating baseline LI. These findings corroborate the hypothesis that α_2 NE receptors have a modulatory role in selective attention. We speculate that noradrenergics, such as guanfacine, may be effective in the treatment of attention deficits.

Introduction

The process of attention and learning requires the ability to select relevant stimuli to which responses may be beneficial, and the ability to disregard those that are not. Accordingly, a stimulus which is a part of the environment and of no consequence to the organism should not result in a response. Conditioning a response to a stimulus that is new and always connected to a certain adverse event occurs faster than conditioning to a stimulus which has previously been experienced as non-consequential. This phenomenon, referred to as Latent Inhibition (LI), has a critical role in attention and learning. LI is often used in animal models because the phenomenon is robust in animals and humans (De La Casa et al. 1993; Gray et al. 2001; Williams et al. 1997).

Dopaminergic antagonists have been the most studied and successful modulators of LI for many years and hence these drugs were considered the major modulators of LI (Moran et al. 1996; Weiner et al. 1996). Amongst the dopaminergic drugs that have been shown to reverse a disrupted LI are haloperidol, clozapine and risperidone (Feldon and Weiner 1991; Moran et al. 1996; Moser et al. 2000). All three of these drugs also show some affinity to the α_2 receptor (Miyamoto et al. 2005). Because many of the previously tested drugs have effects on multiple systems, we were interested in examining the role of NE in LI with a more selective drug.

The importance of the noradrenergic system in attention and alertness has been assessed in many studies (Aston-Jones and Cohen 2005). For instance, when macaque monkeys were trained to respond to a target stimulus embedded in a series of non-target stimuli, Locus Coeruleus (LC, the main source of cortical NE) activity followed correct target tone identification. Interestingly, a high overall activity of the LC correlated with poor performance (Aston-Jones et al. 1999), suggesting a bell-shaped dependence of attention performance with NE activity. Additionally, micro injections of the non-selective α_2 agonist clonidine into the LC of monkeys with poor performance quickly increased attentive focus and performance (Ivanova et al.1997). The relevance of NE in other attention models, as well as the co-affinity for NE receptors that many previously examined Dopaminergic antagonists exhibit, prompted the present study of the NE component of LI.

Previous studies testing LI after norepinephrine depletion following DSP-4 administration may have discouraged further research (Archer et al. 1983; Archer et al. 1986). Those findings concluded that the alteration in latent inhibition related solely to context mismatch differences between conditioning and testing. While the context mismatch condition was not relevant to the present study, the finding that NE down regulation alone produced no effect motivated us to use a different approach. Our study differs in that we use guanfacine in conjunction with the

amphetamine model of disrupted attention to examine the modulator properties NE has on LI. Specifically we tested the hypothesis that NE plays a role in LI by investigating the effect of reducing NE release prior to amphetamine administration.

Administration of the selective α_2 receptor agonist guanfacine (Summers et al. 1981) has been shown to effectively decrease NE release by activating presynaptic autoreceptors (Stark et al. 1989; Stark 2001). Assuming guanfacine down-regulates the NE output into the synaptic cleft, we expected that amphetamine would not be able to significantly up-regulate the effect of the synaptic NE over baseline levels, leading to a normal expression of LI. The choice of guanfacine as test drug was also supported by its possible clinical relevance since the drug is approved by the FDA (Federal Drug Administration) and hence preclinical results would easily translate, as toxicity studies have been completed and the necessary drug doses in humans are well established. More importantly, the selectivity of guanfacine for α_2 receptors, would allow specific conclusions regarding the mechanisms of the hypothesized involvement of NE in attention and learning.

Materials and Methods

Animals:

Male Sprague – Dawley rats (n =58) were housed in groups of 4 or 3 with ad-libitum food in a housing room separate from the testing room until the beginning of the baseline period. The animal facility was temperature controlled and on a 12 h reverse light cycle. At the beginning of the baseline period rats were moved to the testing room and kept on a 20 g per day diet. At the onset of the experiment all rats were between the ages of 2 and 3 months, and between weights 300g and 550g. All experimental protocols were in accordance with the guidelines set

forth by the Commission on Life Sciences, Institute for Laboratory Animal Research (ILAR) and by the Of?ce of Laboratory Animal Welfare (OLAW).

Equipment:

The testing cage was placed in a sound attenuation chamber, which had an internal noise level of 60 db, measured with a commercially available sound level meter (Digital-Display Sound-Level meter Model: 33-2055,Radioshak). Rats were placed inside a 20 cm by 20 cm by 26 cm metal cage with a shocking grid raised 6 cm from the floor. The grid bars had alternating polarity and were 1.1 cm apart. A water spout was available to the rat through a 4 by 4 cm slot in the cage. A photo-gate was placed directly under the drinking spout to record lick patterns. The photo-gate was connected to a USB-1024 digital I/O (Measurment Computing, Norton, MA). The input was recorded through a custom MatLab program (MathWorks, R2010a, Natick, MA).

Drugs

Amphetamine and guanfacine (Sigma, St. Louis, MO) were dissolved in 0.9% saline, and injected interperitoneally. Amphetamine (1mg/kg) was injected 30 minutes before prexposure and 30 minutes before conditioning. Guanfacine (0.3mg/kg) was injected 1 hour before preexposure and 1 hour before conditioning (Sagvolden et. al. 2006, Jakala et. al. 1999). The only difference between the treatments of these groups was on the preexposure day as explained below.

Behavioral Protocol

In order to minimize animal's stress, every rat was handled before the experiment for 2 minutes per day for a period of five days prior to each experiment (Barak et al 2010). Animals were placed on water restriction five days prior to the start of the experiments to ensure motivation for the water licking task (baseline days 1-5). During this time, animals were restricted to ad libitum water access only during the one hour training period each day (7-8 p.m.). The following five days the animals were allowed to drink for 20 min/day inside the testing chamber prior to their 1 hour of drinking per day (baseline day 5-10). In preliminary testing this elaborate baseline period was found necessary to achieve consistent licking patterns across subjects. In order to determine the effect of preexposure of an auditory stimulus on LI, separate groups of preexposed (PE) and non preexposed (NPE) rats were tested under three unique pharmacological conditions: NPE saline (n=7), PE saline (n=9), NPE amphetamine (n=7), PE

amphetamine (n=8), NPE guanfacine plus amphetamine (n=8), and PE guanfacine plus amphetamine (n=9). The LI protocol was performed on days 11-13, and consisted of 3 stages: preexposure on day 11, conditioning on day 12, and testing on day 13, each conducted 24 hours apart.

Pre-Exposure

On day 11, rats were placed in the testing chamber with no water availability. The pre-exposure groups were given 40 target tone presentations (5 second 3 kHz pure tone) at variable intervals between 8 and 40 s. The non-preexposure (NPE) groups were kept in the testing chamber also with no water access for the same amount of time but without hearing the tone presentations.

Conditioning

On day 12, all rats were placed in the testing chamber with water access removed. Five minutes after arrival, they received the first tone-shock pairing (a 1-s long 0.5 mA current, starting directly after the end of the target tone). After another five minutes they received a second tone-shock pairing. Rats were removed from the chamber five minutes following the tone-shock conditioning.

Testing

On day 13, all rats were placed back into the testing chamber, which was now again equipped with the water access. The animals were allowed to drink as they were accustomed to during the baseline acclimation. Licks were recorded. On lick number 90, the target 3 kHz tone started playing continuously and did not stop until the rat was removed from the chamber. Animals conditioned to the tone- shock pairing, stopped licking water and froze momentarily. The time (A) to complete licks number 80 to 89 prior to the tone was recorded and compared to the time between licks 90 and 99 at the beginning of the target tone (time B). We defined as "Suppression Index" (SI) the quotient $A/(A+B)$. A rat which showed a strong reaction to the onset of the tone by stopping licking would display an SI of around 0.2 as seen in fig.1A, while a rat which had no reaction to the onset of the tone had a SI index of 0.5 as seen in fig.1B.

Results were reported as mean ± standard error. The unpaired two-tailed Student t-test was used to assess differences between groups. Differences were accepted as significant if $p < 0.05$ unless otherwise specified. A one-

way ANOVA was also used to determine a drug treatment's relevance to the change in LI. The outliers of used in our calculation for finding Lick Frequencies over the course of each trial were defined by the outer quartile rule. If X is the data point being considered then it must satisfy the following $Q2+(Q2-Q1)*3< X< Q1-(Q2-Q1)*3$ to not be considered a critical outlier. Where Q1 is the median of the lower half of a sorted array containing all data points and Q2 is the median of the upper half. 3 was defined as the factor for a critical outlier by convention. Figure.2 A excludes outliers as defined above when displays the difference in lick frequency from the first 89 licks on last baseline day to the first 89 licks of the testing day.

Results

Baseline

Lick frequencies before and after conditioning were examined for each rat group to identify any differences in their reaction to the shock. The baseline frequencies from each group were within 1 standard deviations of the total sample mean. Fig.2A depicts the group mean change of the lick frequency from the last day of baseline to the testing day. Using one-way ANOVA we examined the relevance of the drug treatment on the change in lick frequency and found no significant influence ($F=0.815$ $p=0.546$, $df=5$). Three independent Student t-tests revealed no significant difference between the NPE and PE groups of any drug treatment. As an additional control measure we verified that there was no significant difference between any treatment groups' times to achieve 1800 licks during the baseline period. Fig.2B depicts the averages in seconds of the total time spent in the chamber prior to the completion of 1800 licks. A one-way ANOVA revealed no influence of the treatment group factor on the time to complete 1800 licks ($F=1.297$, $p=0.283$, $df=5$). Again the three Student t-tests revealed no significant difference between the NPE and PE groups of any drug treatment.

Latent Inhibition

On the testing day LI of all groups was assessed by comparing the association rates between the preexposed and non-preexposed groups. The association rate was measured via the suppression index (SI), as defined in Methods. The control groups confirmed prior findings on saline and amphetamine LI effects. Saline-treated rats previously preexposed to the tone exhibited a decreased rate of association with the foot shock, as seen by the

significant differences in SI found between preexposed and non-preexposed animals (SI = 0.51 ± 0.04,1 n = 9 for PE versus 0.34 ± 0.16 for NPE, n = 7, p = 0.01). Conversely, preexposed rats treated with amphetamine showed no difference with the non-preexposed group. In fact, amphetamine treatment, suppressed the SI difference between the NPE and PE groups (SI = 0.31 ± 0.22, NPE, n = 7, vs. SI = 0.27 ± 0.21 in PE, n = 8; p=0.63), confirming that amphetamine pretreatment attenuates normally occurring Latent Inhibition.

We tested the hypothesis that α_2 adrenoceptors modulated LI by determining the effect of the α_2 agonist guanfacine, injected 30 min before amphetamine administration, on the suppression ratio. Guanfacine significantly blocked the amphetamine effect, restoring SI to the baseline values (saline injection): NPE (SI = 0.34 ± 0.13, n= 8 vs. PE SI = 0.62 ± 0.26, n= 8 in the Guanfacine + Amphetamine group, p=0.02, fig.3).

Discussion

Our finding, that alpha 2 adrenoreceptors play a role in modulation of an amphetamine-impaired attention model, corroborates previous behavioral experiments in coloboma mice. Coloboma mice display higher impulsivity and hyperactivity due to their higher NE concentrations (Jones and Hess 2003). Also coloboma mice show disrupted LI (Bruno et al. 2007) similar to that induced by acute injection of amphetamine (Weiner et al. 1987). Interestingly, LI is restored and hyperactivity is reduced in coloboma mice by inhibiting the NE system with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4). In the case of coloboma mice DSP-4 injections reduced NE to a moderate level similar to those found in sham mice. In our experiments pretreatment with guanfacine elicited a similar effect on amphetamine administration during LI testing.

A cellular mechanism which may explain our observations could be that activation of α_2 receptors by guanfacine produced a presynaptic down regulation of NE output into the synapse (Stark et al 1989; Stark 2001). Such a reduction of NE output prior to the administration of amphetamine could be the cause of our observed reversal of the amphetamine disruption of LI in the presence of guanfacine. This interpretation suggests that NE over activity produces disrupted LI and therefore disrupted selective attention.

These findings are also consistent with those of Aston-Jones and colleagues concerning selective attention (Aston-Jones and Cohen 2005). Our data may be compared to Aston-Jones's NE attention studies because amphetamine

increases NE activity and disruption of LI is linked to inattention. In their study macaque monkeys were distracted while experiencing high levels of NE; our rats had disrupted LI under amphetamine-induced high catecholamine levels. Monkeys experiencing moderate levels of NE activity in the Aston-Jones experiments could be in a similar behavioral state to rats that had been given guanfacine plus amphetamine or saline because both treatments result in moderate levels of NE and good LI expression or good attention performance in the Aston-Jones experiments.

The amphetamine reduction of LI is considered a model for attention deficit in schizophrenia, and it can be reversed by most anti-psychotic drugs clinically used today (Warburton et al. 1994; Moran et al 1996). Typical anti-psychotics are supposed to produce their effect by acting on the dopaminergic system because they have a large affinity for dopaminergic receptors. Our finding that a NE based drug can produce similar results in the LI model as the typical anti-psychotics indicates that the above mentioned affinity to NE receptors many anti-psychotics have should not be neglected when determining the functional component of these anti-psychotic drugs.

Considering the LI model's relevance to schizophrenia, our findings additionally support further investigation of NE based drugs for use on psychosis. One protocol which used guanfacine as an adjunct treatment has already shown some promise in human trials (Friedman et al. 2001)

Conclusion

We have found a link between NE and LI. An up regulation via reuptake inhibition of catecholamines by amphetamine disrupts LI only if there was no prior guanfacine treatment. The effect we produced had been achieved by many other substances before; this was the first time that NE had been shown to play a modulator role in the phenomena. The new link provides motivation for further investigation of adrenergic pharmacology in attention and learning because the ability to ignore irrelevant stimuli is of paramount importance for attention and can be experimentally observed through LI.

Legends to the figures

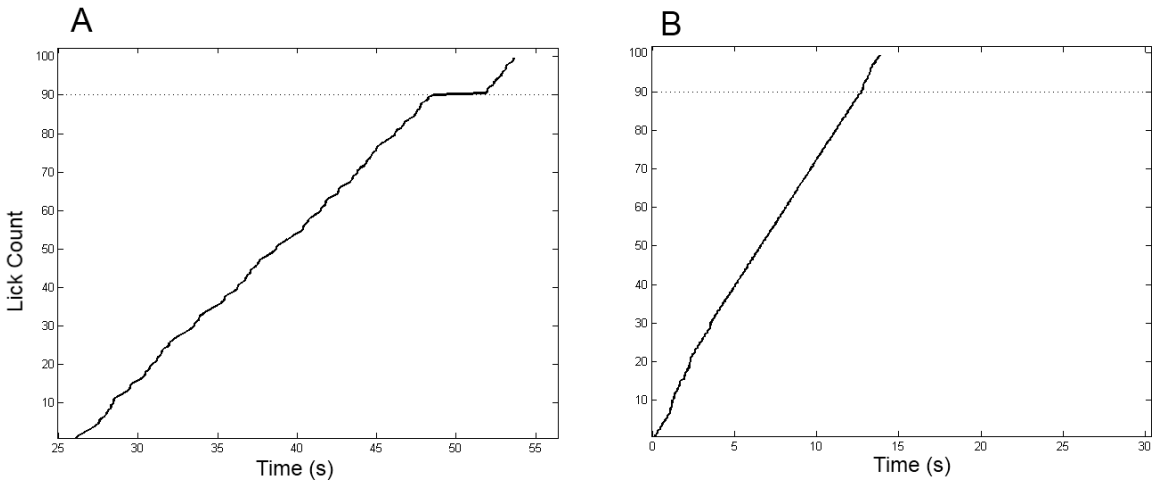

Figure 1. Prototypical licking patterns

A: prototypical licking pattern for a NPE saline treated rat on testing day. This example would give a suppression index of about 0.2, indicating a strong association of the foot-shock with the tone played at lick 90. The suppression index is calculated as *A/(A+B)* where A is the time to complete licks 80 to 89 and B is the time to complete licks 90 to 99. B: prototypical licking patter for a PE saline treated rat on testing day. This example would give a suppression index of about 0.5, indicating no association between foot-shock and tone.

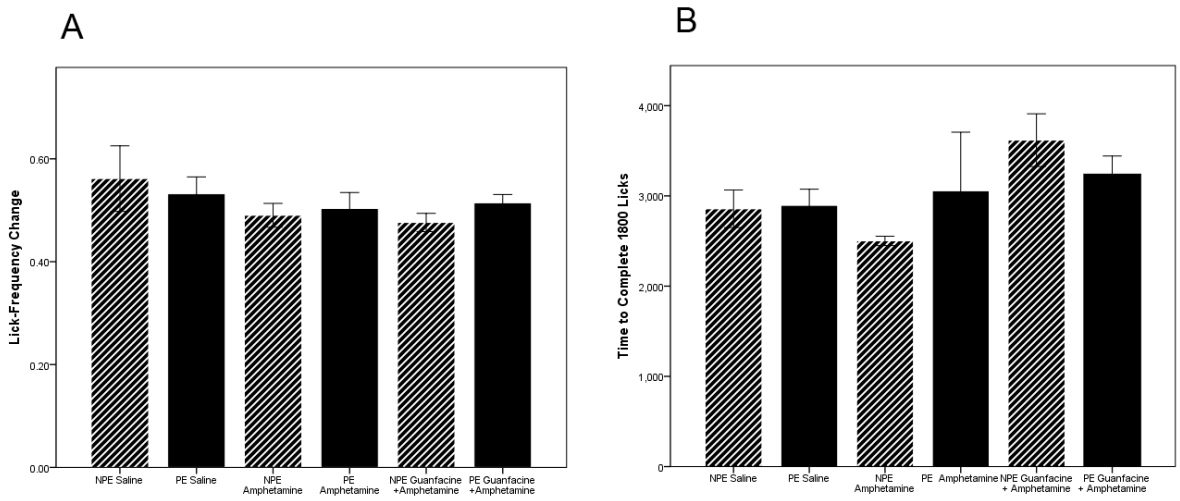

Figure 2. Licking frequency change and mean time to achieve 1800 licks

A: Change in the licking frequency from the last day of baseline to the testing day. Only licks prior to lick 90 were used to calculate the licking frequencies and critical outlier pauses were ignored (See Methods). The differences presented are quantified as *Baseline Frequency/(Baseline Frequency + Testing Frequency)*. B: Mean time to achieve 1800 licks during the baseline period for each treatment group. Error bars represent standard error of the mean (s.e.m.).

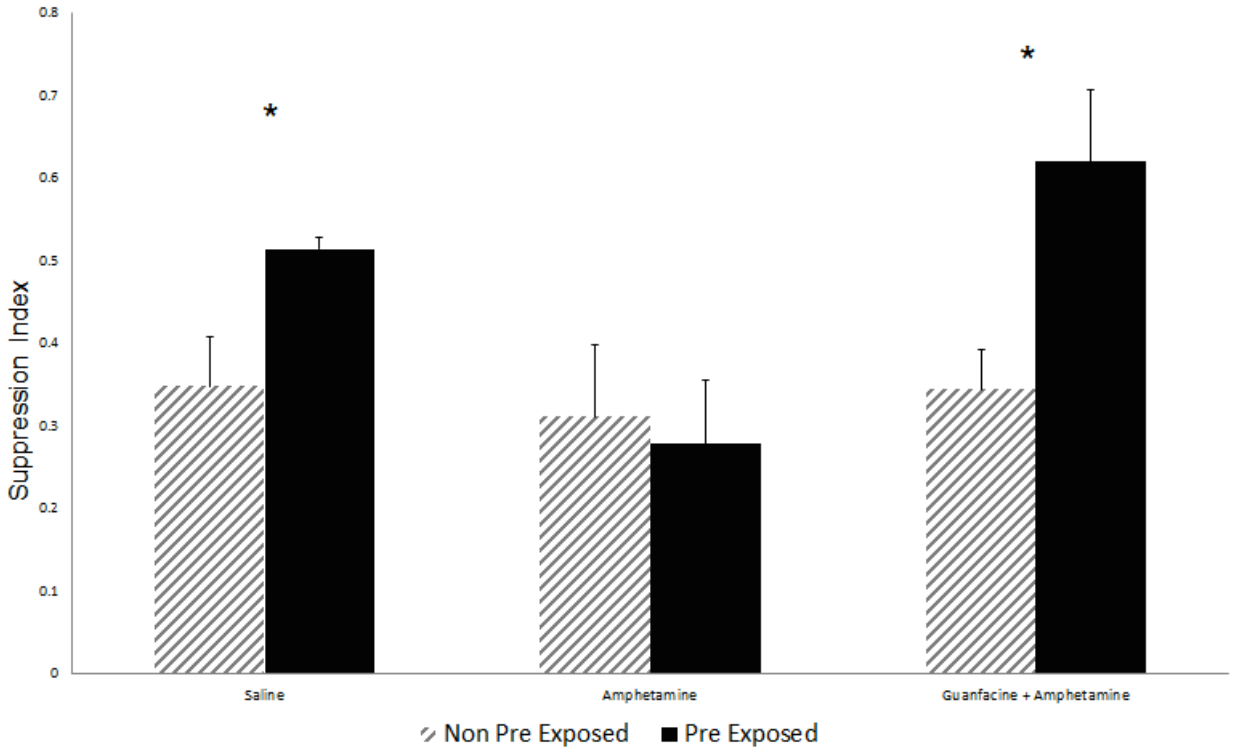

Figure 3. Effects of pharmacological treatment on LI

LI is operationally defined as the difference between the SI (= *A/(A+B)*) of the pre-exposed and the non pre-exposed groups. The difference between the left most pair of bars corresponds to the normal LI for the saline group. Amphetamine pre-treatment blocks LI (middle bars). Guanfacine injection 30 min before amphetamine prevents the amphetamine disruption of LI (rightmost bars), restoring an SI difference similar to the one observed for saline.

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