Dopaminergic Activity in the Medial Prefrontal Cortex Modulates Fear Conditioning

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Abstract- The purpose of the present study was to determine the role of medial prefrontal cortex (mPFC) dopaminergic system in fear conditioning response considering individual differences. Animals were initially counterbalanced and classified based on open field test, and then were given a single infusion of the dopamine agonist, amphetamine (AMPH) and antagonist, clozapine (CLZ) into the medial prefrontal cortex. Rats received tone-shock pairing in a classical fear conditioning test and then exposed to the tone alone. Freezing responses were measured as conditioned fear index. The results showed that both AMPH and CLZ infusion in mPFC reduced the expression of conditioned fear. This finding indicates that elevation or reduction in the dopaminergic activity is associated with the decrease of fear responses, despite preexisting individual-typological differences.

Introduction

The inability to suppress unwanted fear memories is a major problem in many psychiatric disorders like phobia, panic attacks and post traumatic stress disorders. There is increasing evidence supporting the hypothesis that post traumatic stress disorder (PTSD) is associated with extinction failure. The prefrontal cortex has been strongly implicated in fear expression (1,2) and extinction (3,4).

It has been known that fear memory is mediated by projection of infralimbic to hypothalamic and midbrain sites (5). The infralimbic nucleus contributes the majority of mPFC inputs to the central nucleus of the amygdale (5,6), and receives information about stimuli association (7). This part plays a key role in the expression of behavioral and autonomic indices of conditioned fear (6,8).

One of the important neurotransmitters involving in the aversive situation in the mPFC is dopamine. Dopamine is implicated in many behaviors, including motor function, cognition, reward processing and fear responses; however, the role of dopamine in fear processing remains unknown.

Previous studies showed that lesion to the ventral part of mPFC impaired fear extinction (9), but to the dorsal part enhanced acquisition of conditioned fear (6, 9). However, the study of Gregory *et al.*, (2000) (10) suggested that ventral part of mPFC is not necessary for the expression of fear. It is postulated that one of the factors determining the fear response could be typological characteristics (individuality). It is well established that animals exhibit marked differences in behavioral reaction to stimuli (11) based on different neurochemistry and molecular genetics of their brain structures (12-14). To our knowledge the role of mPFC dopamine on fear expression in different typological characteristics has not been studied. To address this issue, we categorized rats into typological subgroups then followed by fear conditioning experiment. Dopamine alteration was carried out by local microinjection of d-amphetamine or clozapine. Since the fear system will respond similarly in humans and rodents (6), we used the Pavlovian classical conditioning, as a method for evaluating fear conditioning. In this method a neutral conditioned stimulus (CS), such as a tone is paired with an aversive unconditioned stimulus (US), usually a foot shock, so

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that subsequent presentation of the CS elicits conditioned fear responses. In order to characterize the relationship between preexisting individual fear and medial prefrontal dopamine alterations on fear memory, this experiment was conducted in two distinct groups based on individual typological characteristics.

Materials and Methods

Subjects

Fifty male Wistar rats weighing 200-250 g were used in this study. Animals were maintained on a 12 hour dark/light cycle. Food and water was proportioned *ad libitum*. All procedures were approved by the Institutional Animal care and use Committee of Guilan University of Medical Sciences.

Open field test

In order to reduce stress, the animals were handled for 3 consequent days for three minutes in experiment room. Each animal was tested separately in an open field apparatus for 5 minutes to evaluate the individual differences using an a digital camera.

Surgery

Animals were anesthetized with sodium pentobarbital 50 mg/kg, and underwent the sterotaxic surgery to insert the guide cannula in the mPFC. Insertion was done according to the standard procedure: briefly, animals were fixed on sterotaxic apparatus and after applying of lidocaine, an incision was made to expose the skull. After finding bregma and lambda in a horizontal level, a pair of guide cannulae (9 mm, 26 gauge, and stainless steel) was implanted through 1.5 mm holes. Cannulae were fixed in the following coordinates from Paxinos and Watson (1998) (15): 3mm anterior, 0.5 mm lateral to bregma and 3.1 mm ventral to skull surface. The guide cannulae were fixed with dental cement for which three small stainless screws, and then stainless stylets (34 gauge) was inserted into the guide cannulae to prevent occlusion. Micro infusions into the mPFC were carried out using 5μl Hamilton syringe connected to a poly-ethylene tube. Rats of group one received bilaterally 0.5 μl d-amphetamine sulfate (AMPH , Sigma) dissolved in normal saline (10 μg per side). The other group received clozapine (CLZ, Sigma) dissolved in saline and small amount of glacial ascetic acid (16 μg per side). The controls group received the same volume of appropriate vehicle. Drug dosages used in our study has been reported to induce behavioral effects without disturbing motor activity or brain damages (15). Immediately after injections rats were placed on fear conditions test box.

Behavioral test

Rats were divided into hyperactive, hypoactive and intermediate groups on the basis of open field test. Thirty two rats from hyperactive and hypoactive groups were cannulated for control and treatment groups. All groups conditioned with two trials of tone-shock for a total time of eight minutes. This experiment consisted of three sessions: conditioning, context and tone test. The conditioning step consisted of two tone-shock trails (30 second, 85 decibel and 2.9 KHz tone followed by one sec, 0.5 mA foot shock). Context test was carried out one day after conditioning, in order to assess persistence conditioning to the place. For this reason, rats were placed in the same box (but without shock) which the day before they were exposed to foot shock. Tone test was conducted two days after conditioning, in order to assess the persistence of conditioned fear to the tone. This part of experiment is carried out in another room, distinct from the conditioning room, and consisted of eight minute persisting tone similar to conditioning. Before and after the test, there were two minute blocks in the shock boxes. On day three, treatment and control rats of both hyperactive and hypoactive rats received bilateral infusion of drugs or vehicle into the mPFC immediately before tone test, and then were placed in the shock box for 8 min. During the tests, the observer was hidden from animal's eyes. Immobility and the behavior of the rat were scored as freezing and freezing was defined as reduction in any movement except respiratory movements.

Analysis of results

Statistical analysis was conducted using the SPSS software system. The total time spent in freezing in the first four min blocks was calculated. For non parametric data Mann-U-Whitney test, and for evaluating parametric data, Student's t-test was used. Level of significance set at $P<0.05$.

Histology

Before drugs infusion, methylene blue was infused into the cannulae in two animals to visualize the cannulae placement. Brains were stored in formalin, sectioned, and analyzed for histological test. In all rats used in experiments, the tips of the infusion cannulae were located within the ventral part of the mPFC. Figure 1 shows the tips of the infusion located unilaterally within the ventral part of the mPFC.
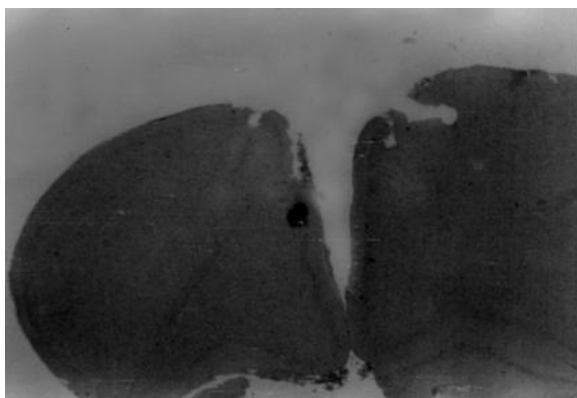
Figure 1. Photomicrograph of a coronal brain section with the tracts of the guide cannula showing the infusion site in the medial prefrontal cortex.

Results

Based on behavioral characteristics determined in the open field test, we classified the animals into three groups. The rats of group one were characterized by less times of movement to the center of open field in comparison with group two ($P<0.001$). They also exhibited more intense horizontal movement at the periphery ($P<0.01$), center ($P<0.0001$), and more exploration activity ($P<0.01$). The number of grooming, defecation and urination in this group were not significantly different ($P=0.6$, $P=0.9$, $P=0.3$ respectively) (Figures 2 and 3).

Thus rats of group one, were regarded as "hyperactive" and those of group two as "hypoactive". The rats of group three were classified as intermediate and were excluded from experiment I, relating to AMPH infusion. The four infusion groups from hyperactive and hypoactive animals were counterbalanced and matched for freezing levels during conditioning and context test and there was no significant differences in freezing between two different behavioral types of rats during conditioning ($P<0.2$).

In experiment I, rats of both hyperactive and hypoactive groups received AMPH (10 µg, n=8) or saline (n=8) into the mPFC immediately before the tone test. Conditioned freezing to the tone previously paired with foot shocks was lower in the AMPH- receiving hyperactive group (19.1 ± 12.2 sec, $P<0.004$) , (Figure 4) ,and also in AMPH- receiving hypoactive group (37.5 ± 19.7 sec, $P<0.01$), than in the vehicle receiving of both hyperactive (98.1 ± 20.9 sec), and hypoactive rat (128 ± 46.7 sec), (Figure 5). No significant differences was observed in fear expression between hyper and hypoactive groups followed by infusion of amphetamine, or vehicle ($P<0.8$).
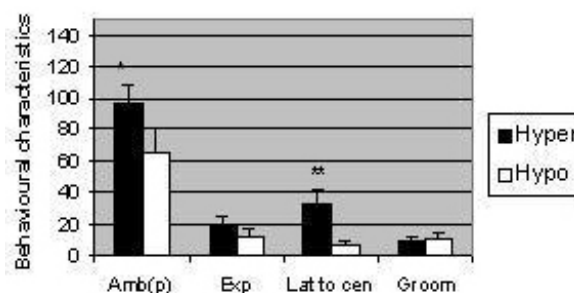

Figure 2. Behavioral characteristics of animals of group 1 (black columns, n=16) and group 2 (white columns, n=16) in the open field test. The horizontal axis shows behavioral measures and the vertical axis shows values of behavioral measures.* $P<0.05$, ** $P<0.001$ compared with rats of group 2. AMB (*P*): number of peripheral sector; EXP: number of excursions into the hole, Lat to cen: times to go to center; Groom: duration of grooming.
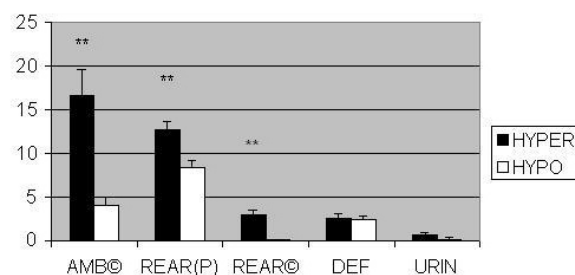

Figure 3. Behavioral characteristics of animals of group 1 (black columns, n=16) and group 2 (white columns, n=16) in the open field test. The horizontal axis shows behavioral measures and the vertical axis shows values of behavioral measures.* $P<0.05$, ** $P<0.001$ compared with rats of group 2. AMB©: number of central sector crossing; REAR(P) number of rearing in peripheral sectors; REAR(C): number of rearing in central sectors; DEF: number of defecations; URIN: number of micturitions.

As shown in Fig 5 clozapine (n=8) infusion into the mPFC of both hyperactive and hypoactive groups caused significant reduction in fear conditioning expression ($P<0.05$). No significant differences was observed in fear expression between hyper and hypoactive groups followed by infusion of amphetamine, or vehicle ($P<0.75$).

The results of this part indicate that infusions of both amphetamine and clozapine before tone test interfere with the expression or retrieval of conditioned fear. Surprisingly drugs, agonist and antagonist decrease the fear responses.
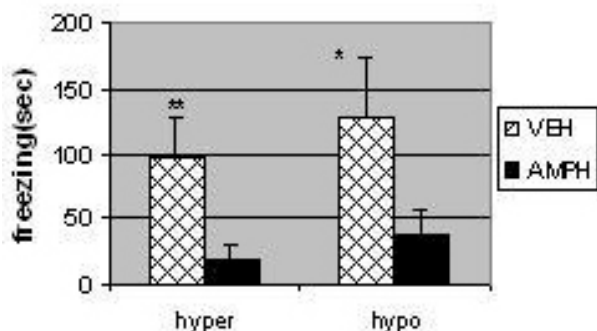
Figure 4. Effects of amphetamine infusion on average freezing during tone test sessions in hyperactive and hypoactive group. Average freezing during tone test sessions expressed as a mean time spent freezing in four first minutes. * $P < 0.05$, ** $P < 0.01$.
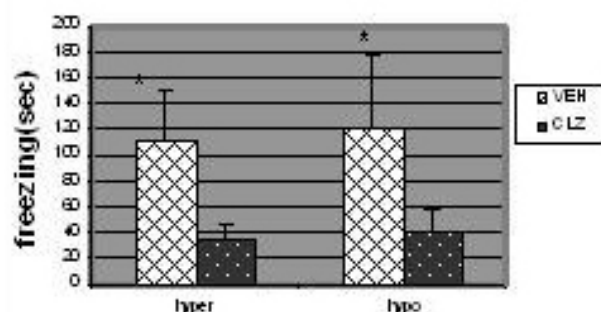

Figure 5. Effects of Clozapine infusions on average freezing during tone test sessions in hyperactive and hypoactive groups. Average freezing during tone test sessions expressed as a mean time spent freezing in four first minutes. * $P < 0.05$.

Discussion

The results of present experiment showed that both hypo- and hyperactive groups differed drastically in their level of emotionality obtained from open field test. Although the hyperactive group showed small freezing in compared to hypoactive, the difference was not significant. Dopamine alteration in mPFC showed that the antagonist, CLZ, and the agonist, AMPH, both reduced the expression of conditioned fear. The reduction in fear conditioned was not attributable to alterations in locomotor activity, because the general activity of animals were monitored and matched immediately after drug infusion in other groups. Therefore reduction in freezing cannot account for hypoactivity, because two distinct groups divided by individual typological characteristics, showed remarkable differences in activity in open field, but they didn't differ in their freezing levels pre conditioning experiment. Moreover, the infusion of AMPH at doses used in our study does not affect sensory processing and

locomotor activity (17). Surprisingly amphetamine receiving hypoactive and hyperactive didn't show significant differences in the expression of conditioned fear. This observation shows that dopamine transmission is enough to override individual differences. Consistent to our result the studies of Angio *et al.* (18), Giorgi *et al.*, (19); Siemiatkowski *et al.*, (20) showed no significant relationship between freezing and open field criteria.

The main finding of the present study is that prefrontal dopamine neurons are involved in modulating the normal retrieval or expression of fear conditioning response. The fact that expression of conditioned fear was not completely inhibited, but only showed reduction, indicate that mPFC has a secondary, rather than primary role in the expression of fear. It seems that physiological process of fear extinction in mPFC following dopamine transmission might strengthen extinction memory, or blocking the retrieval of a learned association between a CS and US despite individual typological characteristics. Our findings support Pavlov's original notion that extinction is a new learning, rather than erasure of conditioning. Although it has been shown that there is a difference in basic dopamine levels in the two hyper and hypoactive group of rats (12), it seems that dopamine transmission overrides this difference. To our knowledge this is the first study to evaluate fear conditioning and dopamine transmission with regard to typological/individual characteristics in rats. Future studies are required to investigate specific role of different dopamine receptors subtypes, in these groups. In conclusion, the present study indicates that the dopaminergic network in the mPFC facilitates the stabilization of fear memory. Elevation or reduction in the dopaminergic activity is associated with the decrease of fear responses, despite preexisting individual-typological differences.

Acknowledgments

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