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GABA, glutamate, dopamine and serotonin transporters expression on forgetting

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ABSTRACT

Notwithstanding several neurotransmission systems are frequently related to memory formation; forgetting process and neurotransmission systems or their transporters; the role of γ-aminobutyric acid (GAT1), glutamate (EACC1), dopamine (DAT) and serotonin (SERT) is poorly understood. Hence, in this paper western-blot analysis was used to evaluate expression of GAT1, EAAC1, DAT and SERT during forgetting in trained and untrained rats treated with the selective serotonin transporter inhibitor fluoxetine, the amnesic drug d-methamphetamine (METH) and fluoxetine plus METH. Transporters expression was determined in the hippocampus (HIP), prefrontal cortex (PFC) and striatum (STR). Results indicated that forgetting of Pavlovian/instrumental autoshaping was associated to up-regulation of GAT1 (PFC and HIP) and DAT (PFC) while SERT (HIP) was down-regulated; no-changes were observed in striatum. Methamphetamine administration did not affect forgetting at 216 h post-training but up-regulated hippocampal DAT and EACC, prefrontal cortex DAT and striatal GAT1 or EACC1. Fluoxetine alone prevented forgetting, which was associated to striatal GAT1 and hippocampal DAT up-regulation, but prefrontal cortex GAT1 down-regulation. Fluoxetine plus METH administration was also able to prevent forgetting, which was associated to hippocampal DAT, prefrontal cortex SERT and striatal GAT1, DAT or SERT up-regulation, but prefrontal cortex GAT1 down-regulation. Together these data show that forgetting provokes primarily hippocampal and prefrontal cortex transporters changes; forgetting represent a behavioral process hardly modifiable and its prevention could causes different transporters expression patterns.

1. Introduction

Forgetting is a cognitive basic ability of the brain, in which our brains are routinely engaged and without it our life would be a chaos. Forgetting could be an unintentional process characterized as a failure to remember or a strategic memorial function that helps to reduce interference in the processing or retrieval of relevant information (Ludowing et al., 2010; Wylie, Foxe, & Taylor, 2008). Forgetting (or retention loss) refers to apparent loss of information already encoded and stored in an individual's long-term memory. It is a spontaneous or gradual process in which old or recent memories are unable to be recalled from memory storage (see e.g., Mansuy, 2005; Wixted, 2004) or failure to encode, maintain or retrieve information as well as memory impairment or a failure to intentionally commit information to memory and to retrieve it when needed. The concept and study of forgetting cover from theories (i.e., cue-dependent forgetting, trace decay, organic causes, interference theories, decay theory), experimental design (e.g., cue-overload (A–B, A–C) learning) to procedures or paradigms (e.g., extinction, reversal learning) (for reviews see Davis, 2010; Mansuy, 2005; Wixted, 2004; Wylie et al., 2008; ;Zhou and Riccio, 1996). Even in some theories forgetting has been hypothesized as the result of various processes: retrieval failure, interrupted consolidation, interference and passive decay (Neath & Surprenant, 2003); being the latter two the more studied. Thornkeke (1913) introduced the "law of disuse" to explain forgetting as a decay of memory over time and McGeoch (1932) opposed to this law, and introduced the concept of retroactive interference (Fioravanti & Di Cesare, 1992). Both hypotheses have received experimental support; however, neither of them has been found strong enough to overrule the other (see Fioravanti & Di Cesare, 1992; Klatzky, 1975).

Certainly, human studies have led to distinguish brain areas involved in intentional and unintentional forgetting, namely parahippocampal gyrus/hippocampus, superior frontal gyrus, posterior cingulated, bilateral inferior parietal and medial parietal cortices (see e.g., Wagner & Davachi, 2001; Wylie et al., 2008). The investigation of molecular basis of forgetting has showed that it appears to depend essentially on protein phosphatases and the formation of memory depends on protein kinases. Memory and forgetting are indeed reciprocally controlled by a balance between kinases and phosphatases that determines the efficacy of learning and the persistence of memory (Mansuy, 2005). Moreover, in the model of

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olfactory memory in flies, independent molecular mechanisms for memory formation and forgetting were found (Shuai et al., 2010). The involvement of memory formation, synaptic plasticity and signal transduction pathways, such as Ca2+, cAMP, and transcription factor CREB-dependent cascades (Davis, 2010; Kandel, 2001; Margulies, Tully, & Dubnau, 2005) as well as expression of membrane receptors and drugs manipulation have been documented (see e.g., Ersche et al., 2011; Pérez-García & Meneses, 2008; Pérez-García, Gonzalez-Espinosa, & Meneses, 2006; Tellez, Rocha, Castillo, & Meneses, 2010). Recent evidence indicates that memory formation, amnesia and anti-amnesic effects modulated transporters expressions (Tellez, Gómez-Víquez, & Meneses, 2011, Tellez, Gómez-Víquez, & Meneses, 2012). Hence, in this work, an attempt was made to explore the role of GABA (GAT1), glutamate (EACC1), dopamine (DAT) and serotonergic (SERT) membrane transporters during forgetting by using a protocol of decay or forgetting (216 h post-training or one week retention interval) in an autoshaping task. Herein, the corresponding transporters were determined in crucial areas for memory, namely the hippocampus (HIP), prefrontal cortex (PFC) and striatum (STR) by western-blot analysis during forgetting. Aiming also, the pharmacological basis of forgetting fluoxetine and meta-amphetamine were used. Fluoxetine (a 5-HT uptake inhibitor) improves memory consolidation and meta-amphetamine (a drug of abuse) impairs it (see e.g., Meneses, Pérez-García, Ponce-Lopez, & Castillo, 2011a, , 2011b). Long-lasting memories are most efficiently formed by multiple training sessions separated by appropriately timed intervals and autoshaping associative learning task has been used previously to detect effects induced by memory, amnesia, drugs and aging (see Meneses et al., 2011a, 2011b; Tellez et al., 2012). Notably, hippocampus mediates declarative memory, striatum mediates stimulus–response "habit" formation (for review see Meneses, 2003; Meneses et al., 2011a, 2011b). Hence, another key aiming of this work is determining the contribution of hippocampus and striatum to autoshaping memory formation. To our knowledge this is the first study aiming to evaluate four different transporters in the same animal in a forgetting protocol, which hopefully will provide a better panorama of brain changes as whole under these cognitive processes.

2. Experimental procedures

2.1. Animals

Adult male Wistar rats (weighing 250–300 g; Pharmacobiology Department, CINVESTAV) were used. Animals were housed at normal temperature (22 ± 1 °C) and light-controlled room under a 12:12 h light–dark cycle (light on at 7:00 a.m.). Food and water were freely available a week before to acclimate animals to the environment. After that period, their body weights were gradually reduced to 85% by reducing the time of food intake during seven days. Thus, to the end of each day of autoshaping training/testing sessions and during the interruption period (see below) of training/testing sessions (one week), trained and untrained animals received access to food during 30 min. All experiments were performed in accordance with the Institutional Review Committee (CICUAL; Project No. 047/02) for the use of animal subjects in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

2.2. Drugs

Drugs used were: fluoxetine HCl (Eli Lilly, Indianapolis) and d-methamphetamine (METH, Pharmacobiology Department, CINVESTAV). All drugs were prepared fresh, dissolved in physiological saline and were injected (i.p) in a volume of 1 ml/kg. Aiming to affect forgetting, animals were given only one administration after the autoshaping fourth training/testing session of fluoxetine (10.0 mg/kg), METH (1.0 mg/kg) and their co-administration (fluoxetine followed by METH administration) and final autoshaping session occurred 216 h later. Autoshaping trained animals were used for the western-blot analysis and were compared with untrained groups.

2.3. Autoshaping learning task

2.3.1. Apparatus

The autoshaping learning task apparatus (Coulbourn Instruments, Lehigh Valley, PA, USA) has been previously reported (see e.g., Tellez et al., 2010, 2012); in short, it included a standard sound-attenuation system and has the following dimensions: 25 cm width, 29 cm in length and 25 cm in height with floor of bars. A retractable lever was mounted 4 cm above the floor and 10 cm from the right and left walls. The lever required a 10 g force for operation. A food magazine was located 5 cm to the right of the lever and 3 cm above the floor. A house light was located in the right top corner and maintained turned on during session period.

2.3.2. Food-magazine training

For habituation period (≈15 min) each rat was placed into an experimental chamber and had access to 50 food-pellets (45 mg each); once the animal presented 150 nose-pokes (measured by a photocell) into the food-magazine and ate all food-pellets, the autoshaping program was initiated.

2.3.3. Autoshaping

Autoshaping training consisted of discrete trials. A trial involves the presentation of an illuminable retractable lever during 8 s (conditioned stimulus, CS) followed by the delivery of a food-pellet (unconditioned stimulus, US) with an intertrials time (ITT) of 60 s (Pavlovian pairing stimulus–stimulus [S–S]). When the animal presented a lever-press response to the CS (Instrumental pairing response-stimulus [R-S]), the lever was retracted, the light was turned off and a food pellet (US) was immediately delivered and the ITT was then begun. The response during CS was regarded as a conditioned response (CR) and its increase or decrease was considered as an enhancement or impairment measure of memory (index of memory), respectively. After a number of such presentations, the animal approaches the CS and presents instrumental responses (conditioned response [CR]), lever-press. If the animal failed to present the CR, the CS lasted 8 s and at the end of this period the US was delivered. Thus animal is exposed to both Pavlovian and instrumental conditioning. CRs were transformed to a percentage of total trials for each session. There were five autoshaping sessions, one for food-magazine training and four for autoshaping training/testing sessions. The autoshaping training session consisted of 10 trials and subsequent STM and LTM training/testing sessions of 20 trials. Animals were tested 1.5 (STM), 24 and 48 h (both LTM) and subsequently at 216 h interruption period (i.e., no autoshaping session) for forgetting period (Fig. 1).

The interruption period for the forgetting protocol was selected by preliminary experiments (data no showed), in which by using independent animals, it was noted that one week of interruption following autoshaping training/testing sessions, animals showed a decreased number of CR% or decreased retention (i.e., forgetting). In this protocol the individual level of CR (i.e., memory) prior the interruption (48 h) was considered the basal level of retention for each individual animal. This interval was selected because previous neurobiological, pharmacological and behavioral evidence (Huerta-Rivas, Pérez-García, González-Espinosa, & Meneses, 2010; Perez-Garcia & Meneses, 2009; Tellez et al., 2012) indicate that this time detects increases and decreases on performance when memory is being consolidated. Also, it should be noted that in this work,