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Research report

Autoradiographic study of serotonin transporter during memory formation

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ABSTRACT

Serotonin transporter (SERT) has been associated with drugs of abuse like D-methamphetamine (METH). METH is well known to produce effects on the monoamine systems but it is unclear how METH affects SERT and memory. Here the effects of METH and the serotonin reuptake inhibitor fluoxetine (FLX) on autoshaping and novel object recognition (NOR) were investigated. Notably, both memory tasks recruit different behavioral, neural and cognitive demand. In autoshaping task a dose–response curve for METH was determined. METH (1.0 mg/kg) impaired short-term memory (STM; lasting less of 90 min) in NOR and impaired both STM and long-term memory (LTM; lasting 24 and 48 h) in autoshaping, indicating that METH had long-lasting effects in the latter task. A comparative autoradiography study of the relationship between the binding pattern of SERT in autoshaping new untrained vs. trained treated (METH, FLX, or both) animals was made. Considering that hemispheric dominance is important for LTM, hence right vs. left hemisphere of the brain was compared. Results showed that trained animals decreased cortical SERT binding relative to untrained ones. In untrained and trained treated animals with the amnesic dose (1.0 mg/kg) of METH SERT binding in several areas including hippocampus and cortex decreased, more remarkably in the trained animals. In contrast, FLX improved memory, increased SERT binding, prevented the METH amnesic effect and re-established the SERT binding. In general, memory and amnesia seemed to make SERT more vulnerable to drugs effects.

1. Introduction

Drugs of abuse like D-methamphetamine (METH) and (+/−)3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") reduce brain serotonin transporter (SERT) density and provoke memory deficits [1–3,88]. Indeed, prior exposure to MDMA significantly diminished the correlation with cortical SERT binding and also in abstinent ecstasy users impaired memory and reduced SERT binding [1]. Importantly, METH has effects on dopamine, noradrenaline and serotonin transporters and receptors (see e.g., [4,5]). Interestingly, the serotonin and dopamine systems interact during memory formation (see [6]). Serotonin or 5-hydroxytryptamine (5-HT) pathways project [7,8] to brain areas implicated in memory, including neocortex and hippocampus [9]. Growing evidence indicates that via multiple 5-HT receptors [10] and SERT, this monoamine plays a role in cognitive processes (see e.g., [11–13]). Notably, the SERT binding is found to be decreased in patients with Alzheimer's disease (AD) into brain areas such as hippocampus, frontal, temporal and entorhinal cortices as well as raphe nuclei [14–19]. Unfortunately, it is unclear if the loss of SERT is related to memory deficits or serotonin reuptake inhibitors (SSRIs) could be useful in the treatment of memory deficits of AD-patients. Notably, pharmacological and genetic manipulations of SERT modify memory performance in several behavioral tasks (see e.g., [13,20–25]). For instance, immediately post-training administration of SSRI like fluoxetine improved memory consolidation in learning tasks such as passive avoidance [26] and autoshaping [27]. In addition, the SERT-deficient and SERT over-expressing mouse [28] and rat models offer excellent new light. For instance, SERT knockout (−/−) rats that received acute tryptophan depletion (ATD) relative to wild-type ($SERT^{+/+}$), both show transiently lowered central serotonin levels and impaired short-term memory (STM; lasting for minutes to hours) in the novel object recognition task (NOR; [29]). As it is unclear whether memory affects SERT or vice versa, hence, in this paper the aim was to investigate, on the one hand the interaction between METH and fluoxetine (FLX) on cognitive functions of rats in two behavioral tests; and on the other hand, the relationship between the binding pattern of SERT in untrained vs. trained injected animals with saline, METH, FLX, or FLX plus METH. Since changes in SERT have also been reported in brain areas linked to amnesia and AD (see above), it seems appropriate to use behavioral tasks involving diverse brain areas and cognitive demand.

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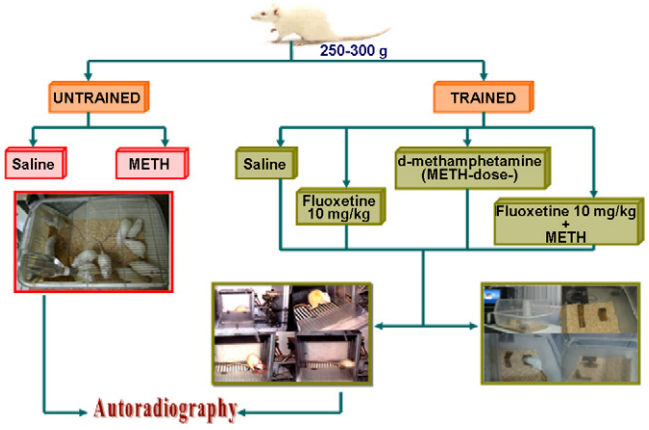

Fig. 1. Experimental design of control and experimental untrained, autoshaping trained and NOR groups.

Many behavioral tasks covering a wide variety of behaviors and processes are subsumed under the banner of 'memory' (see [30–32,91]). Of these tasks, autoshaping [33] and NOR (see below), recruit different behavioral, neural, and cognitive demands (see [12,91]), despite the differences both tasks have been used for assessing NOR STM and/or long-term memory (LTM) (see e.g., [34,35]) and autoshaping STM and LTM (lasting days to months). Research groups have reported that autoshaping modulated the binding of protein 5-HT_{1A} and 5-HT_{2A} receptors in hippocampus and cortex, the dissociation of the orbitofrontal and infralimbic cortex contribution, etc. (for references see [12]). Frequently comparable results of those found in autoshaping have been reported in memory tasks such as passive avoidance, novel object recognition, social recognition, Morris water maze, etc. (for review see [91]). The rationale of choosing autoshaping is that diverse evidence supports the notion that autoshaping is an associative learning task and involves brain areas implicated in declarative/explicit (i.e., hippocampus) and non-declarative/implicit (i.e., striatum) memory ([12,13,36]; however, see [11]). Therefore, the autoshaping task was used to determine a dose–response curve for METH. Next, the autoshaping and NOR were used to test the SSRI fluoxetine (10.0 mg/kg), the drug of abuse METH (effective dose 1.0 mg/kg) and their co-administration (Fig. 1). Fluoxetine dose was based on full dose–response studies (see [23,27]), where a memory facilitation effect was observed. As already mentioned STM lasted less 90 min, no required hippocampal translations or transduction but is dependent on prefrontal cortex mechanisms, LTM is defined as lasting longer that 24 h, requiring hippocampal protein and mRNA synthesis within 6 h following training [22,37,38]. In the NOR task, METH (1.0 mg/kg) only decreased performance of STM, nevertheless, impaired performance of both STM and LTM in the autoshaping task. This indicates that METH had long-lasting effect in the autoshaping task; therefore, new autoshaping trained saline, fluoxetine, METH and fluoxetine–METH groups were used for the autoradiography study. Besides these groups, untrained and trained saline or METH groups were included. Twenty-eight brain areas were selected (see Table 1) since they are important for memory [9,13] and/or present serotonergic projections (see [7]). Finally, as there are differential roles of right and left sides of the brain in memory formation [39] and pharmacological effects [40], herein in order to get some insights in the hemispherical dominance the right vs. the left hemispheres were compared. Notably, hemispheric dominance (i.e., hemispheric specialization) is important in processes such as semantic processes, working memory and LTM (see e.g., [41]). We hypothesized that memory, amnesia and pharmacological manipulation of SERT affect SERT binding and hemispherical dominance. Part of this work was presented in the Society for Neuroscience Meeting [42].

2. Experimental procedure

2.1. Materials and methods

2.1.1. Subjects

The experimental protocol was approved by an 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). Male Wistar rats (12 weeks old) were collectively housed (home cage measuring $60 \times 40.7 = 2442\ cm^2$, allowing $244\ cm^2$ per animal of (300 g bodyweight) in a temperature and light-controlled room under a 12:12 h light–dark cycle (light on at 7:00 A.M.). Water and food were provided ad libitum for autoshaping trained and untrained as well as NOR animals. After that period, as in other experiments [43,92] for the autoshaping trained and untrained animals, their body weights were reduced to 85% by gradually reducing the food intake during 7 days. To the end of each day of autoshaping experiments, untrained and trained animals received access to food during 30 min. Separate animals were used for autoshaping and NOR tasks.

2.2. Autoshaping task

In an autoshaping or sign-tracking setting, a hungry animal is placed in a conditioning chamber to find food pellets (unconditioned stimulus [US]) in the food-magazine and is then given a Pavlovian sequential pairing (stimulus–stimulus [S–S]) of a lighted key [33] or a retractable-illuminated lever (conditioned stimulus [CS]) and food (US). After a number of such presentations, the animal approaches the CS and presents instrumental responses (conditioned response [CR]), such as peck, nose-poke, and contact- or lever-press. It should be noted that for the Pavlovian autoshaping procedure [33] pigeons were exposed to repeated (response-independent) presentations of food after the response key was illuminated momentarily. Importantly, within the continued progress of behavioral task development, a Pavlovian/instrumental (P/I) autoshaping task combines both Pavlovian and instrumental conditioning [36,93] where the presentation of an illuminable retractable lever for 8 s (CS) is followed by the delivery of a food pellet (US) with an inter-trial interval of 60 s. When the animal presents a lever-press response to the CS, the lever is retracted, the light is turned off, a food pellet (US) is immediately delivered and it is considered as a conditioned response (CR). If the animal fails to present the CR, the CS lasts 8 s and in the end of this period the US is delivered. Thus animal is exposed to both Pavlovian and instrumental conditioning.

2.2.1. Apparatus

Operant chambers (Coulbourn Instruments) for rats with standard sound-attenuation were used. Chambers were 25 cm wide, 29 cm long and 25 cm high with a 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. Solid-state programming equipment was used for control and recording (Coulbourn Instruments, Lehigh Valley, PA, USA).

2.2.2. Autoshaping training

Each rat was placed into an experimental chamber and allowed to habituate until the animal found and ate 50 food pellets (45 mg each pellet) and presented 150 head-pokes to collect the food pellet previously placed into the food-magazine. A house light provided general illumination and remained turn on all the session. Immediately thereafter, the autoshaping program began and it consisted in the presentation of an illuminable retractable lever for 8 s (CS), followed by the delivery of a food pellet (US) with an inter-trial interval of 60 s. When the animal presented a lever-press response to the CS, the lever was retracted, the light was turned off, a food pellet was immediately delivered and it was considered as a CR. If the animal failed to present the CR, the CS lasted 8 s and in the end of this period the US was delivered. The increase or decrement in the percentage of CR was considered an index of learning. The autoshaping training session consisted of 10 (lasting (12 min) trials and STM and LTM sessions of 20 (lasting nearly 24 min) trials. Compounds were injected immediately after the autoshaping training session and animals were tested 1.5, 24 and 48 h later. For autoshaping experiments a between subjects design was used. Considering the number of animals used, experiments were carried out in separate cohorts, in which each treatment condition of testing was presented for each one. In order to rule out unrelated factors to memory, several precautions were taken, including the tissue comparison from the animal groups at the time they were simultaneously processed and factors other than memory per se, such as stress, age and weight, were controlled (e.g., by daily handled them, using animals of the same age).

2.2.3. Measurements and analysis

CRs were transformed to a percentage of total trials fro each session. Multiple group comparisons were made using ANOVA followed by the Tukey test. In all statistical comparisons, $p < 0.05$ was used as criterion for significance. The n per group