Subchronic Amitriptyline Influences Open-field Behaviours and Spontaneous Working-memory in Healthy Mice

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Abstract Objective: We assessed the effects of amitriptyline on open-field locomotion, stereotypic behaviours and spatial working-memory in healthy mice. **Method:** Five groups of mice were administered vehicle (distilled water), scopolamine (2 mg/kg), or one of three doses of amitriptyline (5, 10 and 15 mg/kg) for 21 days. Behaviours were assessed after the first and final dose of treatment. **Result:** Administration of the first dose of amitriptyline was associated with enhanced open-field horizontal locomotion, rearing and grooming; with repeated administration, there was suppression of horizontal locomotion and rearing. A dose-related decrease in Y-maze and radial-arm maze spatial working-memory was also observed after repeated administration. Compared to scopolamine, amitriptyline was associated with a significant reduction in open-field behaviours, but a significantly-higher spatial working-memory score. **Conclusion:** Repeated administration of amitriptyline in healthy mice was associated with suppression of locomotion and grooming; working-memory deficits were also observed. However, working-memory task performance is significantly better than the scopolamine group.

Keywords Amitriptyline, Antidepressant, Amnesia, Novelty-induced Behaviours, Working-memory, Scopolamine

amitriptyline. Studies have also reported that amitriptyline is a potent activator of microglia and stimulator of 5'-nucleotidase activity [2]; as well as effective in inhibiting tumour necrosis factor and interleukin-b [3]. More recently, researchers like Flament et al [4] reported the efficacy of amitriptyline in the management of eating disorders. Sim et al [5] and Boles et al. [6] also reported clinical evidence of the effectiveness of amitriptyline in the management of cyclic vomiting syndrome. Generally, the discovery of novel therapies for clinical conditions is a desirable end-point for a number of academic or scientific objectives.

Amitriptyline is a mixed serotonergic and noradrenergic uptake inhibitor with strong anticholinergic and antihistaminergic effects. Amitriptyline prevents the uptake of norepinephrine and serotonin in adrenergic and serotonergic neuron by inhibiting the membrane pump mechanism responsible for amine uptake. This may result in the potentiation of neuronal response; largely because reuptake of these biogenic amines is crucial in the termination of neuronal impulses [7] Amitriptyline has also been reported to potentiate dopamine-induced hyperlocomotion [8], via its effects on dopamine receptors (D₂/D₃) [8, 9]. The interference with the reuptake of norepinephrine and/or serotonin is believed to underlie the antidepressant activity of amitriptyline. However, common adverse-effects (such as blurring of vision, dry mouth, and constipation) which may limit amitriptyline use have been attributed to its anticholinergic effects; while sedation is thought to be a result its histamine receptor blockade activity. For any drug, adverse-effects presenting with behavioural symptoms (sometimes referred to as behavioural-toxicity) has been suggested as an aspect of drug-tolerability which is often overlooked. It has been defined as the extent to which a drug can disrupt abilities crucial to the safe performance of the cognitive and psychomotor tasks of everyday life [10]. An uncontrolled level of behavioural toxicity can be counter-therapeutic in the use of drugs like amitriptyline,

1. Introduction

Amitriptyline is primarily used in the treatment of major depression, anxiety, bipolar disorder and attention-deficit hyperactivity disorder [1]. Repurposing has led to its use in the management of migraines, neuropathic pain, fibromyalgia, and as a sleep-aid in insomniacs; meanwhile, research is relentless in defining new roles for old drugs like

since it may exacerbate cognitive dysfunction associated with depression; thereby reducing compliance.

Amitriptyline's effects on brain neurotransmitters, receptors and biogenic amines [8, 9, 11] have been studied extensively; but usually within the context of its antidepressant or analgesic properties. Drugs with anticholinergic effects e.g. milnacipran and scopolamine are known to impair memory [11, 12]; also, a few studies have reported evidence of memory-impairment [13, 14] following amitriptyline administration. Parra et al [15] reported retrograde amnesia in the passive avoidance task, which was unrelated to anxiety response. However, there is a dearth of information on the general behavioural response to amitriptyline administration (especially in health), using validated animal models like the open-field, Y-maze and/or radial-arm maze.

The objectives of this study were to assess the effects of amitriptyline, at doses in mice that translate to a human equivalent dose of between 0.41- 1.22 mg/kg/day (which falls within the therapeutic dose range in humans, irrespective of gender), on open-field novelty-induced behaviours and spatial working-memory in healthy mice. We also compared the effects of amitriptyline at these doses to those observed with a non-selective anticholinergic and standard amnesic agent (scopolamine) [16]. This was with the view of ascertaining if repeated administration of increasing doses of amitriptyline is associated with significant changes in open-field novelty-induced behaviours and deterioration of working-memory; and how the degree of these changes (if present), is comparable to that of scopolamine. Scopolamine is an atropine alkaloid, and a competitive muscarinic cholinergic receptor antagonist, which induces cognitive deficits [17], and alteration in locomotor activity [18-20] and sleep stages [21]. It is a very potent psychoactive drug validated for its use as a standard/reference drug for inducing amnesia in mammals. The choice of scopolamine as a reference drug is premised on its possession of both cognitive (amnesia) and non-cognitive effects, (locomotor hyperactivity) in rodents [19, 20]; hence, it is suitable as reference in assessing the extent of the anticholinergic effect of amitriptyline. The rationale for the study was to ascertain the neurobehavioural response to amitriptyline, in health. We tested the hypothesis that, repeated oral administration of amitriptyline could significantly influence open field novelty-induced behaviours, Y-maze and radial-arm spatial working-memory in healthy mice.

2. Materials and Methods.

2.1. Drugs

Amitriptyline hydrochloride (Amitriptyline Hcl 25 mg tablet, Densa Pharmaceutical Ltd. Mumbai, India), Scopolamine (Locin® as Hyoscine N-butylbromide, 10

mg/mL, Greenfield Pharmaceuticals Ltd. Jiang Su, China).

2.2. Animal Care

Male, Swiss mice (Empire Breeders, Osogbo, Osun State, Nigeria) weighing 18-20 g each were used for this study. Mice were housed in groups of five, in enriched (transparent plastic cages with exercise ladders, shredded-paper beddings, artificial burrows made of plastic tubes and platforms) cages, located in a temperature-controlled quarters (22-25 degree Celsius) with 12 hours of light daily (lights on at 7.00 a.m.). All animals were fed commercial standard chow (Calories: 29% protein, 13% fat, 58% carbohydrate) from weaning. Animals had access to food and water *ad-libitum*, except during the behavioural tests. All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

2.3. Experimental Method

Fifty mice were randomly assigned into five groups of ten (n=10) mice each. Animals received vehicle (oral distilled water at 10 ml/kg, and an intraperitoneal (i.p) injection at 2 ml/kg) or one of three oral doses of amitriptyline (5, 10 and 15 mg/kg), plus i.p injection of distilled water. A fifth group of animals received i.p. injection of a standard amnesic (scopolamine at 2 mg/kg), plus oral distilled water at 10 ml/kg. Treatments were given daily for 21 days. Doses of amitriptyline were calculated by dissolving weighed quantities in distilled water; and given at a volume of 10 ml/kg. Behavioural tests were conducted after first and last dose of the vehicle, scopolamine or amitriptyline.

2.3.1. Behavioural Testing

Open field novelty-induced behaviours such as locomotion, rearing and grooming were assessed after the initial and final dose of the vehicle, scopolamine and amitriptyline. Behavioural tests were conducted in a quiet room between 10 a.m. and 3 p.m. On each of the test days, mice were transported in their home-cages to the behavioural testing laboratory, and allowed to acclimatise for 30 minutes before administration of treatments. Behavioural tests were conducted in a room lit by white fluorescent light; delivering about 130 Lux at the centre of the arena. Thirty minutes after administration of drug or vehicle, behavioural tests were conducted. Animals were allowed to explore the open-field (for 20 minutes first) and then the Y-maze (5 minutes), after which they were immediately placed in the radial-arm maze (for another 5 minutes). At the beginning of the behavioural tests, each animal was placed in the apparatus and its behaviour videotaped {by a ceiling-mounted digital video camera (SMX-F543B), placed 1.5 metres above the arena} for subsequent analysis. After testing, each mouse was removed from the maze and returned to its home cage, and

all interior surfaces cleaned thoroughly with 70 % ethanol, and then wiped dry. At least, 5 minutes was allowed between the testing of individual animals to ensure that the maze was completely dry, and that dispersal of the residual odour of alcohol had occurred. The behavioural parameters were later scored by two independent observers who were blind to the groupings.

2.3.1.1. Open Field Novelty-Induced Behaviours

A twenty minute-period of the following behavioural states: locomotion, rearing and grooming were observed in the open-field, and scored. The open field apparatus is a rectangular arena made of white-painted wood, measuring 36 x 36 x 26 cm. The floor is made of hard wood and divided by permanent red markings into 16 equal-sized squares. The mice were placed in the centre of the field and covered by a small dome for (5 seconds), which was removed at the beginning of the 20 minutes countdown. Generally, spontaneous motor activity was monitored in the open-field as previously described [22, 23]. Thirty minutes after administration of the vehicle, scopolamine or amitriptyline, each mouse was introduced into the field and the total horizontal locomotion (number of floor units entered with all paws), rearing frequency (number of times the animal stood on its hind legs either with its fore-arms against the walls of the observation cage or free in the air) and frequency of grooming (number of body cleaning with paws, picking of the body and pubis with the mouth, and face- washing actions, indicative of a stereotypic behaviour) within the 20 minute period was recorded.

2.3.1.2. Memory (Y and Radial-Maze)

Y-maze and the radial-arm maze were used to measure general activity and spatial working-memory. Spontaneous alternation behaviour (SAB) was used to measure spatial working-memory. SAB comprises the tendency for rodents to alternate their (conventionally) non-reinforced choices of Y-maze or radial maze arms on successive opportunities. The Y-maze spontaneous alternation has been validated in rodents as a measure of working-memory, general locomotor activity and stereotypic behaviour. Spontaneous alternation was assessed using a Y-maze made of white painted wood with three equally-spaced arms (120°, 41cm long, 15 cm high and 5 cm wide). The floor was also made of hard wood and painted white. Each mouse was placed in one of the arm compartments and allowed to move freely until its tail completely entered another arm. The sequence of arm entries was recorded. An alternation was defined as entry into all three arms consecutively. The number of actual alternations is number of sequential arm entries into the three arms, designated A, B and C. The percentage alternation is calculated as $\{(\text{Actual alternations} / \text{Total arm entry minus two}) \times 100\}$ within a 5 minute period [24, 25].

Spatial working-memory in the radial-arm maze was measured as sequential arm entries before making an error.

The apparatus is made of white painted wood with eight equidistantly spaced arms, each about 33 cm long, 15 cm high and 5 cm wide, all radiating from a small central circular platform. Working-memory was assessed when the rat enters each arm a single time. Re-entry into the arms would result in a working-memory error [25].

2.4. Statistical Analysis

Data was analysed using Chris Rorden's ezANOVA for windows, version 0.98. Hypothesis testing was performed using analysis of variance (ANOVA). We tested the hypothesis that a single or repeated oral administration of amitriptyline could significantly alter open-field novelty-induced behaviours and spatial working-memory in healthy mice. Two-factor ANOVA (one between subject, one within subject) was used to test effects of 2 main factors: treatment (five levels: vehicle, scopolamine, and amitriptyline at 5, 10 and 15 mg/kg) and repeated administration (2 levels: initial and final administration) on behaviours in the open-field, Y-maze and radial-arm maze. Tukey's honest significant difference (HSD) test was used for within and between group comparisons. Results were expressed as mean $\pm$ S.E.M and p values less than 0.05 were considered statistically significant.

3. Results

3.1. Effects of Amitriptyline on Horizontal Locomotion

Figure 1 (upper panel) shows the effect of vehicle (VEH), scopolamine (SCOP) and amitriptyline on horizontal locomotion; measured as the number of lines crossed within a 20 minute period. A two-factor ANOVA assessing the main effect of treatment and/or repeated administration, revealed a significant effect of treatment ($F_{(4, 50)} = 33.06$, $p < 0.001$) and a significant effect of repeated administration ($F_{(1, 50)} = 65.4$, $p < 0.001$), with significant interactions between treatment x repeated administration ($F_{(4, 50)} = 20.1$, $p < 0.001$). Tukey HSD analysis revealed a significant increase in horizontal locomotion with SCOP ($p < 0.001$), and amitriptyline at 5 ($p < 0.001$) 10 ($p < 0.001$) and 15 mg/kg ($p < 0.001$) compared to vehicle, following initial administration. With repeated administration, horizontal locomotion increased significantly with SCOP ($p < 0.001$) and decreased with amitriptyline at 5 ($p < 0.001$), and 10 mg/kg ($p < 0.001$). Compared to SCOP control, horizontal locomotion was significantly higher with amitriptyline at 10 mg/kg ($p < 0.002$) and significantly less with amitriptyline at 15 mg/kg ($p < 0.012$) following initial administration; with repeated administration locomotor activity was significantly less with amitriptyline at 5 ($p < 0.002$), 10 ($p < 0.040$) and 15 mg/kg ($p < 0.020$). Pairwise comparisons of the effect of repeated administration revealed horizontal locomotion

which was significantly less with repeated administration of amitriptyline at 5 ($p < 0.001$) and 10 mg/kg ($p < 0.001$) compared to initial administration.

3.2. Effects of Amitriptyline on Rearing

Figure 1 (lower panel) shows the effect of treatment on rearing, measured as the number of times the animal stood on its hind legs either with its fore-arms against the walls of the observation cage or free in the air, within a 20 minute period. A two-factor ANOVA, assessing the main effect of treatment and/or repeated administration, revealed a significant effect of treatment ($F_{(4, 50)} = 18.10$, $p < 0.001$) and a significant effect of repeated administration ($F_{(1, 50)} = 29.2$, $p < 0.001$), with significant interactions between treatment x repeated administration ($F_{(5, 50)} = 10.12$, $p < 0.001$). Tukey HSD

analysis revealed a significant increase in rearing with SCOP ($p < 0.001$) and amitriptyline at 5 ($p < 0.001$), 10 ($p < 0.001$) and 15 mg/kg ($p < 0.001$) compared to vehicle, following initial administration; while with repeated administration, there was a significant increase in rearing with SCOP ($p < 0.001$) and a significant decrease with amitriptyline at 5 ($p < 0.001$), 10 ($p < 0.002$) and 15 mg/kg ($p < 0.010$). Compared to SCOP control, rearing activity was significantly less with amitriptyline at 5 ($p < 0.001$), 10 ($p < 0.001$) and 15 mg/kg ($p < 0.001$) with repeated administration and showed no significant difference with initial administration. Pairwise comparisons of the effect of repeated administration and initial administration revealed a significantly less rearing activity with repeated administration of amitriptyline at 5 ($p < 0.001$) and 10 mg/kg ($p < 0.001$) compared to initial administration.

Figure 1. Effect of amitriptyline on horizontal locomotion (upper panel) and rearing activity (lower panel). Values are means $\pm$ S.E.M. (* $p < 0.05$ significantly different from VEH, # $p < 0.05$ significantly different from SCOP, ## $p < 0.05$ repeated administration significantly different from initial administration). VEH: Vehicle, SCOP: Scopolamine, AMI: Amitriptyline, number of animals per group-10.

Figure 2. Effect of amitriptyline on grooming behaviour. Values are means $\pm$ S.E.M. (* $p < 0.05$ significantly different from VEH, # $p < 0.05$ significantly different from SCOP, # $p < 0.05$ repeated administration significantly different from initial administration). VEH: Vehicle, SCOP: Scopolamine, AMI: Amitriptyline, number of animals per group-10.

3.3. Effects of Amitriptyline on Grooming

Figure 2 shows the effect of vehicle (VEH), scopolamine (SCOP) and amitriptyline on grooming behaviour, measured as the number of body-cleaning episodes with paws, picking of the body and pubis with mouth and/or face washing actions within a 20 minute period. A two-factor ANOVA assessing the main effect of treatment and/or repeated administration, revealed a significant effect of treatment ($F_{(4, 50)} = 34.2$, $p < 0.001$) and a significant effect of repeated administration ($F_{(1, 50)} = 9.56$, $p < 0.010$), with no significant interactions between treatment $\times$ repeated administration ($F_{(4, 50)} = 0.98$, $p < 0.496$). Tukey HSD analysis revealed a significant increase in grooming with SCOP ($p < 0.001$), amitriptyline at 5 ($p < 0.001$), 10 ($p < 0.001$) and 15 mg/kg ($p < 0.001$) compared to vehicle following initial administration; and a significant increase with SCOP ($p < 0.001$) and amitriptyline at 5 ($p < 0.001$) with repeated administration. Compared to SCOP control grooming was significantly lesser with amitriptyline at 5 mg/kg ($p < 0.001$), 10 ($p < 0.001$) and 15 mg/kg ($p < 0.001$) following initial and repeated administration, Pairwise comparisons of the effect of repeated administration revealed significantly less grooming with repeated administration of amitriptyline at 10 ($p < 0.010$) and 15 mg/kg ($p < 0.011$) compared to initial administration.

3.4. Effects of Amitriptyline on Y-Maze Spatial Working-Memory

Figure 3 (upper panel) shows the effects of vehicle (VEH), scopolamine (SCOP) and amitriptyline on spatial working-memory in the Y-maze, measured as the percentage alternation within a 5 minute period. A two-factor ANOVA assessing the main effect of treatment and/or repeated administration, revealed a significant effect of treatment ($F_{(4, 50)} = 18.9$, $p < 0.001$), and no significant effect of repeated administration ($F_{(1, 50)} = 0.341$, $p < 0.561$). However, there were significant interactions between treatment $\times$ repeated administration ($F_{(4, 50)} = 4.42$, $p < 0.011$). Tukey HSD analysis revealed a significant decrease in spatial memory with SCOP ($p < 0.001$) and amitriptyline at 5 mg/kg ($p < 0.020$) compared to vehicle, following initial administration; while with repeated administration, spatial memory decreased with SCOP ($p < 0.001$), and amitriptyline at 10 ($p < 0.027$) and 15 mg/kg ($p < 0.022$). Compared to SCOP control, spatial memory was significantly higher with amitriptyline at 5 ($p < 0.001$), 10 ($p < 0.001$) and 15 mg/kg ($p < 0.001$) following initial and repeated administration. Pairwise comparisons of the effect of repeated administration revealed no significant difference in spatial working-memory with repeated administration of amitriptyline compared to initial administration.

and 15 mg/kg ($p<0.012$) compared to initial administration.

3.6. Effects of Amitriptyline on Spatial Working Memory (Radial-Arm Maze)

Figure 4 (upper panel) shows the effect of vehicle, scopolamine and amitriptyline on spatial working-memory in the radial-arm maze, measured as arm entry before first error within a 5 minute period. A two-factor ANOVA assessing the main effect of treatment and/or repeated administration, revealed a significant effect of amitriptyline dose ($F_{(4, 50)} = 8.70, p < 0.001$), a significant effect of repeated administration ($F_{(1, 50)} = 8.20, p < 0.001$), and significant interactions between treatment x repeated administration ($F_{(4, 50)} = 4.80, p < 0.010$). Tukey HSD analysis revealed a significant decrease in spatial memory with SCOP ($p < 0.010$) compared to vehicle following initial and repeated administration; and a significant decrease with amitriptyline at 10 ($p < 0.032$) and 15 mg/kg ($p < 0.023$) following repeated administration. Compared to SCOP control, spatial memory was significantly higher with amitriptyline at 5 ($p < 0.001$), 10 ($p < 0.011$) and 15 mg/kg ($p < 0.001$) following initial and repeated administration. Pairwise comparisons of the effect of repeated administration revealed no significant difference in spatial working memory with repeated administration of amitriptyline compared to initial administration.

Figure 3. Effect of amitriptyline on spatial working memory (upper panel) and locomotor activity (lower panel) in the Y-maze. Values are means $\pm$ S.E.M. (* $p < 0.05$ significantly different from VEH, # $p < 0.05$ significantly different from SCOP, $^{\text{p}}$ $p < 0.05$ repeated administration significantly different from initial administration). VEH: Vehicle. SCOP: Scopalamine. AMI: Amitriptyline. number of animals per group-10.

Figure 4. Effect of amitriptyline on spatial working memory (upper panel) and locomotor activity (lower panel) in the radial-arm maze. Values are means $\pm$ S.E.M. (* $p < 0.05$ significantly different from VEH, # $p < 0.05$ significantly different from SCOP, # $p < 0.05$ repeated administration significantly different from initial administration). VEH: Vehicle, SCOP: Scopolamine, AMI: Amitriptyline, number of animals per group-10.

3.7. Effect of Amitriptyline on Radial Arm Maze Locomotor Activity

Figure 4 (lower panel) shows the effect of vehicle, scopolamine and amitriptyline on locomotor activity in the radial-arm maze, measured as total arm entry within a 5 minute period. A two-factor ANOVA assessing the main effect of treatment and/or repeated administration, revealed a significant effect of treatment ($F_{(4, 50)} = 6.68$, $p < 0.003$) and repeated administration ($F_{(1, 50)} = 14.00$, $p < 0.001$), with no significant interactions between treatment $\times$ repeated administration ($F_{(4, 50)} = 1.43$, $p < 0.001$). Tukey HSD analysis revealed a significant increase in total arm entry with SCOP ($p < 0.001$) compared to vehicle, following initial administration; and a significant increase in arm entry with SCOP ($p < 0.001$), and amitriptyline at 5 mg/kg ($p < 0.001$) with repeated administration. Compared to SCOP control, arm entry was significantly less with amitriptyline at 5 ($p < 0.010$), 10 ($p < 0.011$) and 15 mg/kg ($p < 0.001$) following initial and repeated administration. Pairwise comparisons of the effect of repeated administration revealed significantly higher arm entry with repeated administration of amitriptyline at 5 mg/kg ($p < 0.001$) and significantly less with amitriptyline at 10 ($p < 0.041$) and 15 mg/kg ($p < 0.010$) compared to initial administration.

4. Discussion

Pharmacological agents such as amitriptyline are widely prescribed for depression, anxiety and several other disorders [26]. Drugs in this class are known to have various neuropharmacological effects; however, the presence of effects without desired clinical benefits may constitute impediments to therapy. This study set out to assess the effects of amitriptyline on open-field behaviours and working-memory in mice. From the present study, we deduced that repeated administration of amitriptyline causes: (1) a reduction in horizontal locomotion and rearing behaviours, in comparison to vehicle (2) a dose-related reduction in grooming frequency compared to scopolamine (3) and a dose-related deterioration in working-memory compared to vehicle.

The results of initial administration of amitriptyline showed an inverted-U response with lesser increases in horizontal locomotor and rearing activities at the extremes (5 and 15 mg/kg), and a greater increase at the median dose compared to vehicle; however, with repeated dosing there is a blunting of this response. A fairly-similar response is observed when horizontal locomotor activity is compared to scopolamine control. A previous study had shown that in the elevated plus-maze, acute amitriptyline administration

caused a significant dose-dependent reduction in entries into closed arms; which is considered a reliable measure of general activity [15]. The locomotor response observed with amitriptyline exceeded that of scopolamine at the median dose, after initial administration. Scopolamine had been reported to induce locomotor hyperactivity via effects on nigrostriatal dopaminergic neurons [27]. The higher locomotor activity associated with amitriptyline at 10 mg/kg would suggest the possibility of an influence on the dopaminergic system. Amitriptyline is known to inhibit the re-uptake of serotonin and noradrenalin, and it also increases the regional turnover of dopamine in parts of the brain that are crucial for locomotion [28]. Also, studies have shown that amitriptyline potentiates the effects of D₃ dopamine receptors in the nucleus accumbens [8]. Dopamine pathways (D₁-like (D₁ and D₅) and D₂-like (D₂, D₃, and D₄) [29] play a role in mediating motor behaviours including locomotion and stereotyped behaviours. Numerous studies have shown that stimulation of the D₃ receptors are inhibitory to both spontaneous and psychostimulant-induced locomotion, as opposed to concurrent D₁ and D₂ receptor-mediated effects [30-33]. The differences in response observed after initial and repeated administration of amitriptyline could be the result of drug exposure-dependent alteration in D₃ receptor stimulation; with lesser modulation after initial administration and increased modulation with repeated administration. It is however worthy of note that some studies came to a different conclusion [8, 34]. Therefore, the behavioural response of D₃ receptor stimulation has been conflicting; with studies reporting an increase in spontaneous locomotor activity in D₃ receptor mutant mice [35-37], but not in others [38, 39]. The overall effect seen with repeated administration of amitriptyline could be attributed to a time-dependent down-regulation by D₃ receptors of the transmission at postsynaptic D₁ and D₂ class receptors, which jointly control motor behaviours [35].

Grooming is a complex and centrally-controlled component of rodent behavioural repertoire which is known to serve several functions; and is regulated by multiple areas in the brain with influences from several neuromediators, neurotransmitters [40] and hormones [41]. In this study, amitriptyline was associated with an increase in novelty-induced grooming behaviours at all doses after initial administration, and an increase at the lowest dose with repeated administration. Increased grooming is an expected behavioural change after introduction of rodents to novel environments [42]; it is attributed to a stress response. Studies have reported a differential role of dopamine in the regulation of grooming behaviour; the activation of D₁ receptors induces intense grooming [43], which is inhibited by D₂ receptors [44]. The GABA system also plays a role in the expression of novelty-induced grooming via GABA_A and GABA_B receptors [45]. GABA_A [46] and GABA_B [47] receptor agonists have been reported to cause a decrease in grooming. Recent studies have also reported that repeated but not initial administration of amitriptyline stimulates

GABA_B receptors in mice [48]; this could be responsible for the significant reduction in grooming observed after repeated administration of amitriptyline. Serotonin is another neurotransmitter that modulates grooming activity. Amitriptyline's inhibition of serotonin reuptake results in an increase in serotonin concentration in the brain [11]. Stimulation of GABA release is mediated by 5-HT₂ [49] and 5-HT₃ [50] receptors; this in itself may also be responsible for the effects of amitriptyline on grooming.

Effects of antidepressants on memory have been linked to their neuropharmacological properties via interactions with cholinergic, histaminergic, serotonergic and noradrenergic neurotransmitter systems, which have strong links in modulation of learning and memory. In this study, scopolamine had an amnesic effect in the Y-maze and radial-arm maze, which is consistent with previously reported effects of scopolamine administration in mice. Amitriptyline (compared to vehicle) showed a dose-related spatial working-memory effects which ranged from no significant difference with vehicle, to a significant decrease in working-memory, irrespective of the memory paradigm used. Compared to scopolamine however, we saw better memory scores with amitriptyline at all doses studied in either the Y-maze, or radial-arm maze. The spatial working-memory task is a function of ambulatory activity. In this study, locomotor activity in the Y and radial arm maze decreased compared to either scopolamine or vehicle at certain doses, it did not (in our own view) impact on the results of the spatial memory tasks, because enough spontaneous alternations were made by each animal to make a statistically significant inference.

Effects of amitriptyline (like many other antidepressants) on memory have been considered as mere side-effects of its use [51]; therefore, there is a dearth of information on the effect of amitriptyline on memory. In this study, the result of amitriptyline's effects on spatial memory corroborates that of a number of studies that have reported impairment of memory following amitriptyline administration in rodents [52-54]. Even though this study differs from previous ones in the memory model employed (radial-arm maze and Y-maze versus the inhibitory avoidance paradigm [15, 55, 56], the results were however comparable. A number of other studies have however reported that administration of amitriptyline was associated with memory improvements, usually in aged mice [55, 57, 58]. The age-related memory response has been attributed to a reduction in stress-related hormone levels and anxiety [55]. There are suggestions that amitriptyline's effects on memory are linked to both its anticholinergic and antihistaminergic effects [13]. Amitriptyline-induced memory-impairment is consistent with reports that drugs with anticholinergic effects cause memory-impairment [59]; however, the modulatory role of histamine (which could either cause an improvement or a deterioration of memory) is both task and brain-region specific [60]. Studies have reported task-associated memory deficits with amitriptyline [13]. The differences in memory

response observed after initial and repeated administration of amitriptyline could be the result of drug exposure-dependent alteration of synaptic plasticity via modulation of long-term potentiation (LTP) or long-term depression (LTD). A few studies have observed memory-impairing effect of repeated amitriptyline use which was absent with single-dose administration, suggesting the influence of duration of administration on memory response [61]. There are suggestions that amitriptyline's ability to disrupt long-term potentiation (LTP) in the hippocampus [54] is a major mechanism for its long-term effect on memory. Alterations in synaptic plasticity have also been associated with deterioration in memory [62]. In this study, mice explored the radial-arm after they had explored the Y-maze; and we observed that amitriptyline administration resulted in dose-related spatial-memory deficit, consistently in both models (Y-maze and radial-arm maze). The radial-arm maze involves a recognition-memory element [63], which would mean that prior exposure to a similar maze (Y-maze) should cause a significant improvement in memory response in the radial-arm maze. However, the observation of deterioration in memory with amitriptyline could also imply a failure of recognition memory and not only spatial working-memory. Compared to scopolamine, memory test performance that were observed in both mazes after amitriptyline administration was an improvement; this suggests that at the doses tested, amitriptyline might not have caused a complete muscarinic receptor blockade, or the influence of other neurotransmitters/neuromodulators may be limiting the memory deterioration [13].

5. Conclusions

Oral administration of amitriptyline causes significant dose-related alterations in behaviours (open-field and spatial working-memory) in healthy mice. Considering its ever-increasing potential clinical applications, this study therefore provides an insight into the degree of cognitive and locomotor disability that may be associated with long-term amitriptyline therapy.

Conflict of Interest

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

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