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Repeated lysergic acid diethylamide in an animal model of depression: Normalisation of learning behaviour and hippocampal serotonin 5-HT$_2$ signalling

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Abstract

A re-balance of postsynaptic serotonin (5-HT) receptor signalling, with an increase in 5-HT$_{1A}$ and a decrease in 5-HT$_{2A}$ signalling, is a final common pathway multiple antidepressants share. Given that the 5-HT$_{1A/2A}$ agonist lysergic acid diethylamide (LSD), when repeatedly applied, selectively downregulates 5-HT$_{2A}$, but not 5-HT$_{1A}$ receptors, one might expect LSD to similarly re-balance the postsynaptic 5-HT signalling. Challenging this idea, we use an animal model of depression specifically responding to repeated antidepressant treatment (olfactory bulbectomy), and test the antidepressant-like properties of repeated LSD treatment (0.13 mg/kg/d, 11 d). In line with former findings, we observe that bulbectomised rats show marked deficits in active avoidance learning. These deficits, similarly as we earlier noted with imipramine, are largely reversed by repeated LSD administration. Additionally, bulbectomised rats exhibit distinct anomalies of monoamine receptor signalling in hippocampus and/or frontal cortex; from these, only the hippocampal decrease in 5-HT$_2$ related [^{35}S]-GTP-gamma-S binding is normalised by LSD. Importantly, the sham-operated rats do not profit from LSD, and exhibit reduced hippocampal 5-HT$_2$ signalling. As behavioural deficits after bulbectomy respond to agents classified as antidepressants only, we conclude that the effect of LSD in this model can be considered antidepressant-like, and discuss it in terms of a re-balance of hippocampal 5-HT$_2$/5-HT$_{1A}$ signalling.

Keywords

Serotonergic hallucinogen, lysergic acid diethylamide, serotonin 5-HT$_{2A}$ receptor, antidepressant, animal model, olfactory bulbectomy, avoidance learning, hippocampus

Introduction

Lysergic acid diethylamide (LSD) is a serotonergic hallucinogen known to induce profound alterations of the human consciousness (Hintzen and Passie, 2010). When abused in an unsupervised context, hallucinogens can have detrimental effects on the individual (Cohen, 1960; Strassman, 1984); when used in a controlled environment, however, they might be of medical value (De Lima Osório et al., 2011; Grob et al., 2011; Winkelman and Roberts, 2007). Although early and extensively recognised for an ability to facilitate certain strategies of psychotherapy (Passie, 1997; Unger, 1964), particularly in the context of anxiety neuroses and/or depressive reactions (Mascher, 1967; Savage et al., 1973), the therapeutic potential of serotonergic hallucinogens has hardly been considered pharmacologically, i.e. in terms of their receptor profile (Montagne, 2007; Riedlinger and Riedlinger, 1994; Vollenweider and Kometer, 2010). Sharing the indolethylamine moiety of the serotonin molecule (Kang and Green, 1970), LSD is a suitable ligand for a variety of monoaminergic, notably serotonin (5-HT) receptors; with low-nanomolar affinity, for instance, it binds to 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (Roth et al., 2002). Both receptor subtypes regulate a variety of functions critically involved in the pathogenesis of depression; the pyramidal integration of excitatory input to the prefrontal cortex (PFC) (Araneda and Andrade, 1991), the hypothalamic-pituitary-adrenal axis (Osei-Owusu et al., 2005; Zhang et al., 2002), as well as the hippocampal neurogenesis and/or cell proliferation (Banasr et al., 2004). In accordance with their functional relevance, long-term treatment with diverse-class antidepressants has been shown to downregulate 5-HT$_{2A}$ receptors in the frontal cortex, and to increase the responsiveness of hippocampal 5-HT$_{1A}$ receptors in a time frame consistent with their delayed therapeutic onset (Gray and Roth, 2001; Haddjeri et al., 1998; Szabo and Blier, 2001). As repeated LSD, acting as an agonist at both receptor subtypes, also downregulates 5-HT$_{2A}$, but not 5-HT$_{1A}$ receptors (in areas, such as the frontal cortex or the hippocampus) (Buckholtz et al., 1985, 1990; Gresch et al., 2005), one might expect it to re-balance the postsynaptic 5-HT signalling in a

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way similar to antidepressants. And indeed, given that cross-tolerance between hallucinogens and antidepressant-class drugs develops (Bonson et al., 1996; Goodwin et al., 1984; Lucki and Frazer, 1982), a mechanistic overlap seems plausible. Challenging this idea of a mechanistic overlap, we here evaluate whether LSD exerts antidepressant-like effects within an established animal model of depression. In the forced swim test, an animal model that responds to one-time antidepressant application, LSD fails to act antidepressant-like (Gorka et al., 1979). Thus, in line with our assumption that 5-HT_{2A} regulation (which requires a repeated LSD regimen) (Buckholtz et al., 1985, 1990) is important for an antidepressant-like effect to occur, an animal model responding to repeated antidepressant treatment might be of more validity. From the few animal models, which meet such a criterion, we have selected here the olfactory bulbectomy because it is the only one considered highly reliable and specific (Cryan et al., 2002; Jesberger and Richardson, 1985). Following the bilateral dissection of the olfactory bulbs, rodents show a variety of behavioural disturbances, such as stress-associated hyperlocomotion or avoidance learning deficits, which reliably ameliorate in response to the (sub-)chronic, but not acute application of drugs specified as antidepressants (Kelly et al., 1997; Song and Leonard, 2005). The bulbectomy induced hyperlocomotion is considered to be of dopaminergic origin (Masini et al., 2004) and might model symptoms of agitated depression. Avoidance learning deficits, on the other hand, involve the serotonin system (Cairncross et al., 1979; Garrigou et al., 1981; Ögren 1986) and appear to have more general implications for the human situation. According to cognitive theory, depression primarily arises from biases in cognitive processing, including attention and memory, which as a consequence corrupt emotional integrity (e.g. Mathews and MacLeod, 2004). As (serotonergic) antidepressants are thought to act on these biases, rather than on mood itself (Harmer, 2008; Harmer et al., 2009), avoidance learning deficits of bulbectomised rats seem to be an optimal proxy for depressive-like cognition biases and their responsiveness to the 5-HT-related action of antidepressant-class drugs.

Thus, for evaluating the antidepressant-like action of LSD, we here repeatedly apply the hallucinogen to bulbectomised rats and investigate the effect on avoidance learning and forebrain 5-HT_{1A}/5-HT_2 signalling. As LSD, despite having high affinity, is not selective for 5-HT_{1A} and 5-HT_{2A} receptors (Roth et al., 2002), we additionally investigate its effect on beta, overall 5-HT, dopamine and noradrenaline signalling. Methodologically, we use the conditioned pole-jumping paradigm and radioligand binding techniques, respectively.

Methods and materials

Animals and housing

For experiments, male Wistar rats (average (Ø) 400 g) (HsdCpb:WU; Harlan Winkelmann, Germany) were used. The animals were housed in groups of five each cage, and held under controlled laboratory conditions (temperature 20±2°C, air humidity 55–60%, light/dark cycle 12:12 (light on at 06:00.)) with standard food pellets (TEKLAD Global Diet, Harlan-Teklad, UK) and tap water ad libitum. All experiments conducted complied with the regulations of the National Act on the Use of Experimental Animals (Germany), as approved by the Tierschutzkommission Sachsen-Anhalt.

Bilateral olfactory bulbectomy

At the age of seven weeks, rats were bulbectomised as described by Grecksch et al. (1997). In brief, animals were anaesthetised with pentobarbital (40 mg/kg, intraperitoneal, 10 ml/kg injection volume) and fixed in a stereotactic instrument. The scalp was incised at the midline, and two holes (Ø 2 mm) were drilled into the skull (one above each olfactory bulb (6.5 mm anterior to bregma, 2 mm lateral to midline)). The bulbs were cut and gently removed by aspiration. The resulting cavities were filled with haemostatic sponges (Gelitaspon, Gelita Medical, The Netherlands), and the skin was closed by tissue adhesive (Histoacryl, Braun Aesculap AG, Germany). Extent and adequacy of the surgical ablation were assessed after decapitation at end of the behavioural experiments. Sham-operated rats were treated alike (including piercing of dura mater), except that their bulbs were not removed.

Behavioural experiments

Treatment. Lysergide[(R,R)-tartrate]-anhydrate (THC Pharm, Germany) was applied for a period of 11 days, once every 24 h (0.13 mg/kg, subcutaneous, dissolved in isotonic saline, 10 ml/kg). Treatment started five days before the behavioural experiments, and continued till 24 h before decapitation. The dose chosen was extrapolated from literature as adequate for activation of 5-HT_{2A} receptors (as indexed by the occurrence of wet dog shakes) (Bedard and Pycock, 1977). The five days beforehand regimen was chosen so to allow 5-HT_{2A} (down-)regulation to precede the behavioural experiments (Buckholtz et al., 1990). To avoid interference from LSD's acute effects (Bignami, 1972; Domino et al., 1965; Schmidt, 1963; Taeschler et al., 1960), administration was performed two hours after each test session (Castellano, 1979). Control animals received saline injections without LSD.

Assignment of rats to conditions (sham/saline vs sham/LSD; bulb/saline vs bulb/LSD) occurred in a randomised fashion.

One-way active avoidance learning (pole-jumping test). Eight weeks after surgery, on the sixth day of subchronic treatment, pole-jumping experiments were set in. On five days in a row, within 10 trials each day, rats had to learn to actively avoid electrical foot stimuli (unconditioned stimulus (US)) by jumping onto a pole. Every trial started with a sound from a buzzer (80 dB) (conditioned stimulus (CS)) which, from the fourth second onwards, was accompanied by the electrical foot stimulation (delivered through stainless steel rods of the test apparatus' floor, and adjusted to the rat's individual pain sensitivity (0.2–0.4 mA)). A trial was restricted to 20 s, but stopped earlier when a rat successfully jumped onto the pole. CS and US overlapped and were co-terminated. The intertrial-interval was stochastically varied (30–90 s). All five sessions were performed at about the same time during the light period. On the first day, rats were allowed five minutes for exploration of the test apparatus, on the following days only one minute was granted. For evaluation of learning, the numbers of successful escapes (instrumental

reactions, ≤20 s) and avoidances (conditioned reactions, ≤4 s) were recorded.

Neurochemical experiments

5-HT$_{2A}$ receptor binding. Twenty-four hours after the last treatment, rats were decapitated, brain regions of interest (frontal cortices and hippocampi) were removed and frozen in liquid nitrogen. For measuring ketanserin-sensitive [^{3}H]spiroperidol binding to 5-HT$_{2A}$ receptors, thawed tissue was homogenised. Cell membranes were pelleted by centrifugation (10 min, 50,000×g, 4°C), washed in Tris buffer (pH 8.0), and resuspended in incubation buffer (50 mM Tris-HCl, containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 50 nM d-butaclamol (D_2 receptor mask) (Sigma-Aldrich, Germany), pH 8.0). Aliquots of the crude membrane suspension (150–250 µg protein) were incubated for 30 min at 37°C with [^{3}H]spiroperidol (specific activity: 800 GBq/mM (Perkin-Elmer, USA)). The membrane fraction was then collected on GF/A grade glass-fibre filters, washed with buffer (50 mM Tris-HCl, pH 8.0), and taken for liquid scintillation counting in a toluene-containing scintillation cocktail. Specific binding was calculated by subtracting non-specific binding (as seen in presence of 0.25 nM [^{3}H]spiroperidol and 1 µM unlabelled ketanserin (Sigma-Aldrich, Germany)) from total binding (obtained with 0.25 nM [^{3}H]spiroperidol alone), and expressed in fmol per mg of protein (as determined by the Lowry Method).

[^{35}S]-GTP-gamma-S binding. For measuring G-protein coupling by 5-HT$_{1A/2}$, dopamine, and (beta) adrenergic receptors, tissue was homogenised in Tris buffer (50 mM Tris-HCl, 1 mM ethylene glycol tetraacetic acid (EGTA), 10 mM ethylene diamine tetraacetic acid (EDTA), pH 7.4) and pelleted by centrifugation. After resuspension in assay buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, pH 7.4), aliquots containing 15–20 µg protein were incubated with 3 µM guanosine diphosphate (GDP) and 0.05 nM [^{35}S]-GTP-gamma-S (specific activity: 46.3 TBq/mM (Perkin-Elmer, USA)) in the presence and absence of the relevant agonist (1 h, 30°C) (10 µM alpha-methylserotonin (alpha-MS for 5-HT$_2$), 100 µM 8-hydroxy-2-[di-n-propylamino] tetralin [8-OH-DPAT for 5-HT$_{1A}$], 100 µM isoprenaline (for beta), 10 µM serotonin, 100 µM dopamine, and 10 µM noradrenaline (Sigma-Aldrich, Germany)). Incubation was terminated by rapid filtration, filters were rinsed in washing buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EGTA, pH 7.4), and taken for liquid scintillation counting of bound radioactivity. Total [^{35}S]-GTP-gamma-S binding was corrected for unspecific binding (in the presence of 10 µM unlabelled GTP-gamma-S), and expressed as E_{max}, % stimulation over basal specific binding.

All determinations were performed at least in duplicate.

Statistical analysis

A two-factor analysis of variance (ANOVA) with repeated measures on one factor (mixed model) was conducted to assess main effects and interaction of *time* and *group* in avoidance learning, and followed by pairwise contrast analysis. Intergroup differences in specifically bound radioactivity were analysed using nonparametric Mann-Whitney U-tests (a-priori planned comparisons). Calculations were carried out using SPSS and GraphPad Prism software. Statistical significance was assumed if the null hypothesis could be rejected at the 0.05 probability level.

Results

Behavioural experiments

The omnibus *F*-test revealed significant main effects for both factors, *time* ($F_{(4,124)}$=69.04, p=0.000 (conditioned); $F_{(4,124)}$=43.22, p=0.000 (instrumental)) and *group* ($F_{(3,31)}$=6.39, p=0.002 (conditioned); $F_{(3,31)}$=2.93, p=0.049 (instrumental)), and a significant *time*×*group* interaction for conditioned reactions ($F_{(12,124)}$=2.62, p=0.004). Results were further probed by pairwise comparison with a-priori specified contrasts. As can be seen in Figure 1, sham-operated rats showed good progress in learning instrumental and conditioned avoidance behaviour. Irrespective of treatment, they rapidly learnt to avoid and/or to escape from the aversive foot stimuli (sham/saline vs sham/LSD: $F_{(1,17)}$=0.08, p=0.78 (conditioned); $F_{(1,17)}$=0.963, p=0.34 (instrumental)). Saline-treated bulbectomised rats failed to achieve the level of performance shown by the sham-operated controls; the acquisition of both, the conditioned and instrumental reactions, was disturbed (sham/saline vs bulb/saline: $F_{(1,14)}$=13.15, p=0.003 (conditioned); $F_{(1,14)}$=4.85, p=0.045 (instrumental)). The repeated administration of LSD, however, led to a normalisation of conditioned avoidance learning: LSD-treated bulbectomised rats caught up with the sham-operated controls (sham/saline vs bulb/LSD: $F_{(1,16)}$=2.16, p=0.16), and significantly differed from their saline treated counterparts (bulb/saline vs bulb/LSD: $F_{(4,56)}$=2.6, p=0.045) (Figure 1 (a)). As to the instrumental reactions, LSD-treated bulbectomised rats did not significantly differ from the sham-operated controls (sham/saline vs bulb/LSD: $F_{(1,16)}$=0.813, p=0.38), the difference from the saline treated bulbectomised animals, however, failed to achieve statistical significance (see Figure 1 (b)) (bulb/saline vs bulb/LSD: $F_{(4,56)}$=0.766, p=0.55).

Neurochemical experiments

5-HT$_{2A}$ receptor binding. As shown in Figure 2, bulbectomy slightly increased the ketanserin-sensitive [^{3}H]spiroperidol binding in hippocampus. This trend of increase (sham/saline vs bulb/saline: u=4, p=0.095) was partially counteracted by the repeated LSD treatment. Although the difference between LSD and saline treated bulbectomised rats fell short of significance (bulb/saline vs bulb/LSD: u=6, p=0.063), the difference between LSD treated bulbectomised rats and saline treated, sham-operated controls was not significant either (sham/saline vs bulb/LSD: u=11, p=0.46). As opposed to its decreasing effect in bulbectomised rats, repeated LSD treatment did not affect the hippocampal [^{3}H]spiroperidol/ketanserin binding of the sham-operated animals (sham/saline vs sham/LSD: u=11, p=0.46). In the frontal cortex, bulbectomy had no significant effect on the ketanserin-sensitive [^{3}H]spiroperidol binding (sham/saline vs bulb/saline: u=9, p=0.27); LSD, however, induced a significant increase (sham/saline vs sham/LSD: u=0, p=0.002) (Figure 2).

[^{35}S]-GTP-gamma-S binding. In the hippocampus, bulbectomy led to a significant reduction in alpha-MS stimulated guanine nucleotide exchange (sham/saline vs bulb/saline: u=5, p=0.041)

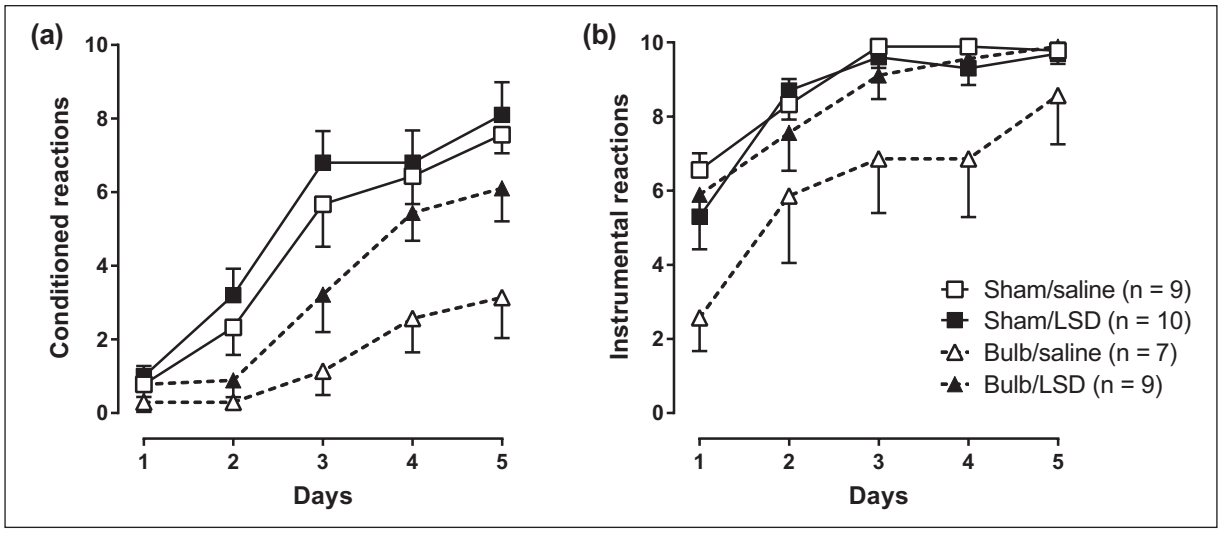

Figure 1. Effect of bulbectomy and repeated lysergic acid diethylamide (LSD) application on conditioned (a) vs instrumental (b) pole-jumping learning over five consecutive days (mean±standard error of mean (SEM)): Repeated measures analysis of variance (ANOVA) with pairwise contrast analysis revealed significant differences between sham/saline and bulb/saline ($F_{(1,14)}$=13.15, p=0.003 (a); $F_{(1,14)}$=4.85, p=0.045 (b)), bulb/saline and bulb/LSD ($F_{(4,56)}$=2.6, p=0.045 (a)), but not between sham/saline and bulb/LSD ($F_{(1,16)}$=2.16, not significant (n.s.) (a); $F_{(1,16)}$=0.813, n.s. (b)). Bulb: bulbectomised rats; sham: sham-operated rats.

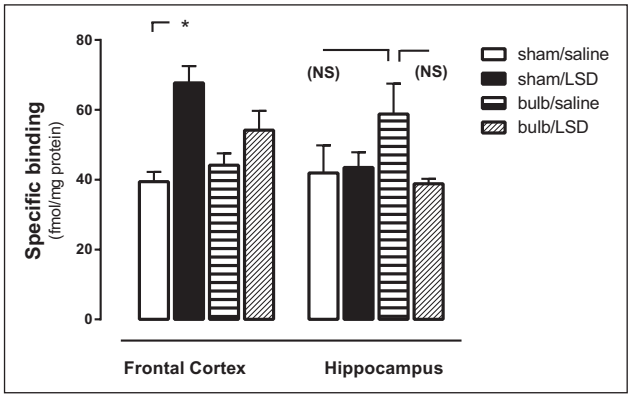

Figure 2. Effect of bulbectomy and repeated lysergic acid diethylamide (LSD) application on specific ketanserin-sensitive [^{3}H]spiroperidol binding to frontocortical and hippocampal membranes. Note the trends of bulbectomy to increase hippocampal 5-HT_{2A} binding, and of LSD to counteract it. Mean + standard error of the mean (SEM) (n=4–6); comparison of groups of interest, *p<0.05. NS refers to a non-significant trend (p<0.10). Bulb: bulbectomised rats; sham: sham-operated rats.

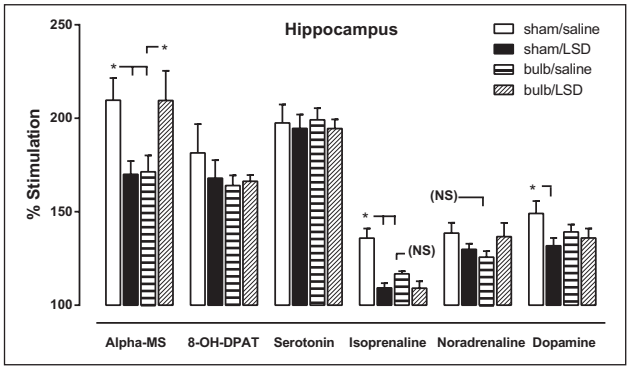

Figure 3. Effect of bulbectomy and repeated lysergic acid diethylamide (LSD) application on [^{35}S]-GTP-gamma-S binding to hippocampal membranes stimulated by various agonists (% of basal binding). Note that from the bulbectomy associated anomalies, LSD selectively normalised 5-HT_2 signalling (as induced by alpha-MS). Mean+standard error of the mean (SEM) (n=4–6); comparison of groups of interest, *p<0.05. NS refers to a non-significant trend (p<0.10). Alpha-MS: alpha-methylserotonin; 8-OH-DPAT: 8-hydroxy-2-[di-n-propylamino] tetralin; bulb: bulbectomised rats; sham: sham-operated rats.

which was reversed by subchronic LSD (bulb/saline vs bulb/LSD: u=6, p=0.032) (Figure 3). In contrast to its resensitising effect in bulbectomised rats, LSD caused a desensitisation of alpha-MS stimulated [^{35}S]-GTP-gamma-S binding in the hippocampus of the sham-operated animals (sham/saline vs sham/LSD: u=3, p=0.0015). Other significant effects and/or trends of bulbectomy, such as the hippocampal decrease in isoprenaline and noradrenaline stimulated receptor signalling (sham/saline vs bulb/saline: u=0, p=0.004; u=2, p=0.057), or the frontocortical increase in alpha-MS, 8-OH-DPAT, and isoprenaline induced [^{35}S]-GTP-gamma-S binding (sham/saline vs bulb/saline: u=4, p=0.026; u=2, p=0.016; u=1, p=0.036) were not reversed by LSD (Figures 3 and 4). The hippocampal signalling stimulated by 8-OH-DPAT, serotonin, and dopamine was neither influenced by bulbectomy (sham/saline vs bulb/saline: u=15, u=18, and u=8, respectively, n.s.), nor by its interaction with repeated LSD (bulb/saline vs bulb/LSD: u=14.5, u=15.5, and u=16, n.s.) (Figure 3). Finally, in the frontal cortex of the sham-operated animals, LSD led to a sensitisation of all receptors investigated, including 5-HT_2 (sham/saline vs sham/LSD: u=3.5, p=0.022) (Figure 4).

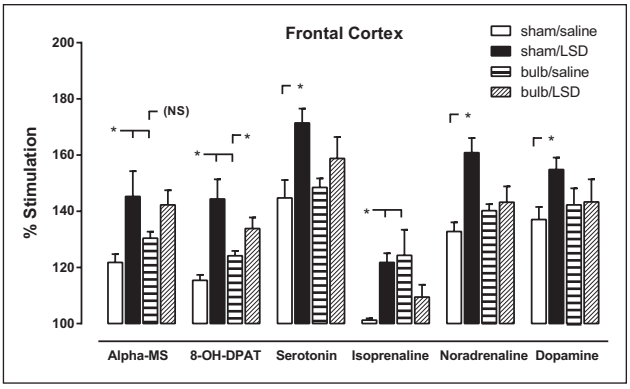

Figure 4. Effect of bulbectomy and repeated lysergic acid diethylamide (LSD) application on [^{35}S]-GTP-gamma-S binding to frontocortical membranes stimulated by various agonists (% of basal binding). Note that from the bulbectomy associated anomalies, none was normalised by LSD. Mean+standard error of the mean (SEM) (n=4–6); comparison of groups of interest, *p<0.05. NS refers to a non-significant trend (p<0.10). Alpha-MS: alpha-methylserotonin; 8-OH-DPAT: 8-hydroxy-2-[di-n-propylamino] tetralin; bulb: bulbectomised rats; sham: sham-operated rats.

Discussion

Exploratory evidence suggests that serotonergic hallucinogens – when psychotherapeutically embedded – might be of assistance in the treatment of neurotic-type depression (Mascher, 1967; Savage et al., 1973), or emotional distress associated with advanced stages of cancer (Grob et al., 2011; Kurland et al., 1973). However, as their acute effects on affection are highly variable and critically dependent on the pre-existing mood (Katz et al., 1968; Metzner et al., 1963), hallucinogens should not be (mis-)conceptualised as acute mood-enhancers or antidepressants in a literal sense. Instead, they might rather be seen as a tool for psychotherapy to facilitate access to emotion-salient cognitions (e.g. memory) and work on the inherent biases that negatively prime the patient's affective mindset (compare Carhart-Harris et al., 2012; Harmer, 2008; Kurland et al., 1973). Here, we refer to the idea that hallucinogens – in a similar way to that hypothesised relevant for repeated antidepressant treatment (Gray and Roth, 2001; Harmer, 2008; Savitz et al., 2009) – might affect mood-relevant cognitive biases by regulation of 5-HT$_{1A/2A}$ receptors. We repeatedly applied LSD to bulbectomised rats, and tested its effect on depressive-like avoidance learning deficits and forebrain 5-HT$_{1A/2}$ signalling. In keeping with former findings (Cairncross et al., 1979; Gebhardt et al., 2013; Marks et al., 1971; Thomas, 1973;), we confirm that bulbectomised rats are deficient in active avoidance learning. Similarly as we noted earlier with imipramine under comparable experimental conditions (Grecksch et al., 1997), or as noted by other labs with amitriptyline or trazodone (Cairncross et al., 1973; Otmakhova et al., 1992), repeated LSD treatment – in a dosage known to induce 5-HT$_{2A}$ related wet dog shakes (Bedard and Pycock, 1977) – largely reverses this deficiency. As the avoidance learning deficits after bulbectomy are reversible by drugs classified as antidepressant only (Kelly et al., 1997), we infer that LSD's behavioural effect in this model can be considered antidepressant-like. Our inference is strengthened by the fact that LSD specifically helps bulbectomised, but not sham-operated, rats.

In addition, we show that bulbectomised rats exhibit various anomalies of monoamine receptor signalling, with 5-HT$_{1A}$, 5-HT$_2$ and beta signalling being sensitised in the frontal cortex, and the latter two being desensitised in the hippocampus. From the given anomalies, the desensitisation of hippocampal 5-HT$_2$ signalling, as indicated by a decrease in alpha-MS stimulated [^{35}S]-GTP-gamma-S binding, is the only one to be normalised by subchronic LSD. Despite alpha-MS being a mixed 5-HT$_{1/2}$ agonist (Ismaiel et al., 1990) rather than selective for 5-HT$_2$ receptors, we think 5-HT$_2$ receptors might be more specifically implicated, because neither bulbectomy nor its interaction with LSD significantly influences hippocampal 5-HT$_{1A}$ signalling. Also, the relevance of hippocampal 5-HT$_{2A}$ receptors might be inferred from our finding that bulbectomy is associated with trends for increased ketanserin-sensitive [^{3}H]spiroperidol binding, and LSD to counteract it. Although these trends should be interpreted with caution, yet they are reminiscent of former findings about bulbectomy upregulating and/or antidepressants downregulating hippocampal 5-HT$_2$ receptors (Earley et al., 1994; Gurevich et al., 1993). Hippocampal 5-HT$_2$ anomalies might be a consequence of the bulbectomy induced raphe degeneration (Nesterova et al., 1997), and the (associated) reduction in local serotonin (Van der Stelt et al., 2005). Remarkably, similar to that seen for avoidance learning deficiency, LSD's (counter-)regulatory action on 5-HT$_{2(A)}$ receptors is specific for the pathological condition; in sham-operated animals, it desenitises alpha-MS signalling, and leaves ketanserin-sensitive [^{3}H]spiroperidol binding unaffected.

LSD exhibits high 5-HT$_{1A}$ and $_{2A}$ affinity, but it is not selective for these receptors. In fact, it binds to a variety of monoamine receptors (Roth et al., 2002), with beta and D$_4$, for instance, complementing 5-HT$_{2A}$ in LSD's behavioural profile (Marona-Lewicka et al., 2009; Mittman and Geyer, 1991). As neither bulbectomy nor LSD's interaction with bulbectomy, however, affects overall dopamine signalling, and LSD normalises hippocampal 5-HT$_2$, but not beta signalling, we think it is reasonable to discuss the LSD induced normalisation of avoidance learning in terms of a re-balance of hippocampal 5-HT$_2$ vs 5-HT$_{1A}$ signalling. Deficits in avoidance learning as well as their reversal by antidepressants have been linked to 5-HT$_{2A}$ receptors (Broekkamp et al. 1980; Gurevich et al. 1993; Ögren 1986), and LSD is known to affect learning via hippocampal 5-HT$_{2A}$ regulation (Romano et al., 2010). Bulbectomy leads to deficient hippocampal neurogenesis, and to an upregulation of brain-derived neurotrophic factor (BDNF) (Hellweg et al., 2007; Jaako-Movits and Zharkovsky, 2005). Although generally considered antidepressant-like, too much BDNF might be detrimental and compromise avoidance learning (Croll et al., 1999). As a model of LSD's antidepressant-like activity one could, therefore, hypothesise that LSD (by activating 5-HT$_{1A}$ and resensitising 5-HT$_2$ signalling) might re-balance the anti-BDNF effect of 5-HT$_{2A}$ against the neurotrophic effect of 5-HT$_{1A}$ receptors (Santarelli et al., 2003; Vaidya et al., 1999). Consequently, a more coordinated turnover of hippocampal neurons might occur, allowing the stress-integration system of bulbectomised rats to more effectively meet the demands of avoidance learning (compare Sairanen et al., 2005; Surget et al., 2011). This model is speculative, however, and needs further investigation. Also, to more clearly establish the role of 5-HT$_{2A}$ and 5-HT$_{1A}$ receptors, future research might co-apply selective antagonists with LSD, combine a selective 5-HT$_{1A}$ with a selective 5-HT$_{2A}$ agonist, or use selective dual agonists instead.

As the latter seem sparse (Ray, 2010), the repeated combination of two agents will raise pharmacokinetic problems, and 5-HT_{2A} antagonists act antidepressant-like themselves (e.g. Otmakhova et al., 1992), though, such a study might be complicated.

Intriguingly in the frontal cortex of the sham-operated rats, LSD significantly increases all binding parameters investigated (including those of 5-$HT_{2(A)}$), which in bulbectomised animals – for the most part – cannot be found. Likewise in the hippocampus, desensitisation of 5-HT_2 and dopamine signalling specifically occurs in the sham rats. Our results contrast with the notion that LSD selectively downregulates 5-HT_{2A} receptors (Buckholtz et al., 1985, 1990). Yet, possibly varying with application scheme, strain and/or embedding of the rats into behavioural procedures, hallucinogens might provoke a more or less complex pattern of receptor regulation (e.g. 5-HT_{1A} downregulation for psilocybin, $alpha_1$ upregulation for DOI, or regional 5-HT_{2A} down- vs upregulation for DOM) (Buckholtz et al., 1988, 1990; Doat-Meyerhoefer et al., 2005). The fact that LSD – despite regulating their neurochemistry – does not affect avoidance learning of the sham rats, underlines that our application scheme was well chosen. Repeatedly applying LSD – such as noted for antidepressant-class drugs – might have counteracted the neurochemical imbalance induced by bulbectomy (including hippocampal 5-HT_2 signalling), thus, normalising the learning capacity (or re-shifting the cognitive bias) of the bulbectomised rats. For the sham animals, in contrast, there had never been such an imbalance (or bias), and the only (or most likely) way in which LSD might have affected their avoidance learning would have been by acutely interfering with learning. Applying LSD two hours after each learning session, however, we minimised the chance of such interference (compare Castellano, 1979; Frieder and Allweis, 1982). Therefore, the LSD-induced changes of the sham rats' neurochemistry might rather be unspecific and (temporally) unrelated to the processes involved in avoidance learning.

In summary, our data demonstrate that in bulbectomised rats, repeated LSD treatment reverses depressive-like avoidance learning deficits, possibly engaging a re-balance of hippocampal 5-HT_2 (vs 5-HT_{1A}) signalling. Given the postulated interrelation between the reversal of mood-relevant cognitive biases and 5-$HT_{(2A)}$ receptor regulation (Harmer, 2008), our findings might have implications for the understanding of how hallucinogens alleviate emotional distress, such as that seen in advanced-stage cancer.

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Conflict of interest

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