Pharmacology Biochemistry and Behavior, Vol. 62, No. 2, pp. 323–327, 1999

0091-3057/99 $–see front matter

PII S0091-3057(98)00157-9

Effects of SCH23390 and Raclopride on Anxiety-Like Behavior in Rats Tested in the Black-White Box

CARRYL TIMOTHY, BRENDA COSTALL AND JAMES W. SMYTHE

Neuropharmacology Research Group, Department of Pharmacology, University of Bradford, Bradford, United Kingdom BD7 1DP

Received 19 May 1998; Revised 4 August 1998; Accepted 4 August 1998

TIMOTHY C., B. COSTALL AND J. W. SMYTHE. *Effects of SCH23390 and raclopride on anxiety-like behavior in rats tested in the black-white box.* PHARMACOL BIOCHEM BEHAV **62**(2) 323–327, 1999.—Dopamine (DA) systems are activated by stress, and this response has as a corollary the induction of stress-related behaviors such as anxiety. In mice, D_2 receptor blockade produces an apparent anxiogenic effect, although locomotor impairments might have been present. We investigated the effects of D_1 and D_2 antagonists on a variety of anxiety-like behaviors induced by the black-white box in rats and carefully screened for any locomotor deficits. Adult male Lister hooded rats were injected with either the D_1 antagonist SCH23390 (0, 0.1, or 0.25 mg/kg IP) or the D_2 antagonist raclopride (0, 0.05, or 0.10 mg/kg IP) 20 min prior to being placed into the white chamber of the black-white box (n = 8–10/group). Rats were videotaped and the tapes were scored for latency to exit the white chamber, latency to reenter the white chamber, time spent in the white chamber, intercompartmental crossing, and locomotor activity. ANOVA revealed no effect of the D_1 antagonist SCH23390 on any behavioral measure. However, the raclopride-treated rats left the white area sooner than control rats ($p < 0.01$). Raclopride-treated rats also exhibited delayed reentry times to the white chamber compared to control rats ($p < 0.01$) and spent significantly less time in the white chamber ($p < 0.05$). Neither SCH23390 nor raclopride affected locomotor activity in a manner that confounded these behaviors. These results confirm that D_2 receptor blockade enhances anxiety in rats tested in the black-white box. © 1999 Elsevier Science Inc.

Anxiety Stress Dopamine Hippocampus Rat Black-white box SCH23390 Raclopride

THERE has been long-standing interest in the effects of stress on central neurotransmission and reciprocal interest in the actions of transmitter systems on the perception/initiation of stress responses. Stressful stimuli produce a thoroughly characterized activation of the hypothalamic–pituitary–adrenal (HPA) axis, culminating in the enhanced synthesis and secretion of adrenocorticotrophin (ACTH) and corticosterone (CORT) into the systemic circulation (3,12,22). The HPA axis is, in part, controlled by a negative-feedback mechanism in the form of brain and pituitary CORT receptors that produce an inhibitory signal on further HPA axis activity.

Recent findings suggest that ventral tegmental area (VTA) dopamine (DA) neurons respond to stress, a finding with some theoretical and practical implications. Initial reports, based on postmortem analysis by of acutely stressed rats, showed elevated DA turnover/utilization, principally in prefrontal cortex (PFC), with comparatively little activation in nucleus accumbens (NAcc) (21,26,34). Other researchers using microdialysis techniques (1,5,10) demonstrated that mesolimbic DA activation was present, but the magnitude of the response varied with stress intensity and detection method employed. There is general agreement that both meso-PFC and meso-NAcc DA projections respond to stress, as measured by increased extracellular DA levels, an effect more pronounced in the PFC compared to NAcc. Moreover, CORT has been shown to stimulate stress-induced DA release, suggesting a functional relationship between DA and HPA activities (14,20).

The significance of increased DA utilization during stress is largely enigmatic, although there are some obvious behav-

Requests for reprints should be addressed to James W. Smythe, Department of Pharmacology, University of Bradford, Bradford, West Yorkshire BD7 1DP, UK.

ioral changes that accompany brief periods of stress. In a previous report, we examined the effects of exogenous CORT administration on anxiety-like behavior (ALB) assessed by the black-white box test. We found that doses of CORT, designed to mimic stress-induced levels, increased ALB in a time-dependent fashion, with the most efficacious pretreatment time at 5 min (33). This result was somewhat surprising, because previous research using the social interaction test (13) and elevated plus maze (2) had shown CORT to be anxiolytic. These differences are difficult to reconcile, but may be due to differential activation of CORT receptor subtypes (31). Given that DA activity increases during stress, it is not surprising that psychotrophic agents that target DA function have also been shown to modulate ALB. For instance, Simon et al. (28) reported that the D_2 antagonist sulpiride increased anxiety as measured by delayed times for mice to move from a black to white compartment. Moreover, the D_1 antagonist SCH23390 blocked the effect of sulpiride, suggesting that the anxiogenesis produced by sulpiride might be the result of increased DA release caused by the antagonism of D_2 autoreceptors. As support for this idea, Simon et al. (29) reported that the DA agonists GBR12783 and RU24926 both produced an anxiogenic response in mice. Taken together, these data would suggest that the DA activation accompanying stressful conditions (i.e., placement into the black-white box) contributes to an animal's emotional state and alters behavior accordingly. However, these data must be interpreted cautiously because only one behavior was examined, and changes in locomotor activity may well have affected the results, as these authors note in their reports. Interestingly, Feenstra et al. (11) have shown that the increase in PFC levels of DA following a mild stressor are blocked by pretreatment with diazepam, a finding that supports the contention that DA activation is central to the expression of anxiety.

The anxiolytic profiles of DA agents administered to rats have received relatively less attention. We have recently provided evidence that the black-white box test does generalize to rats as well as mice (23,30,31), although in rats it may well be comparatively more sensitive to anxiogenic rather than anxiolytic agents. The following study was undertaken to provide further corroboration of the test validity of the black-white box model of anxiety in rats, and to assess the effects of DA antagonists on ALB. Moreover, the earlier reports by Simon et al. (28,29) reported only limited behavioral measures of anxiety and failed to take account of any motoric disturbances that might have affected the dependent variables. We have examined extensive behavioral profiles of our subjects and monitored locomotor activity.

METHOD

Subjects

Adult male, Lister Hooded rats weighing between 350 and 450 g, served as experimental subjects. These were obtained from the breeding unit of the University of Bradford and maintained on site until testing. Rats were housed in groups of five in clear polycarbonate cages, with wood chip bedding material. Cage maintenance was undertaken twice weekly, but never on the day of testing. Food (standard rat chow) and tap water were provided ad lib. The housing room was climate controlled with 60% humidity and temperatures of approximately 22°C. Rats were housed under normal light cycle (on at 0800 and off at 2000 h). Testing was conducted during the lights-on period, and always starting at 1200 h.

Black-White Box Apparatus

The black-white box was similar to that previously employed in our laboratory (6,7), but built slightly larger to accommodate the increase in size of rats over mice. The overall dimensions of the box were 50 × 30 × 30 cm (length, width, height). The bottom of the box was dissected by lines creating a grid appearance consisting of 10-cm squares. The box was further divided into two chambers—the black (20 × 30 × 30 cm), and the white (30 × 30 × 30 cm)—by a barrier possessing a doorway (10 × 10 cm) through which rats could traverse. The black compartment was illuminated with red lights, while the white compartment was intensely illuminated by bright white lights. A video camera, connected to a VHS recorder and monitor was used to record each rats activities in the box. The entire black-white box was completely circumscribed by a heavy, black curtain to minimize any possibility of distraction created by experimenter movement or ambient room lights. The testing room was isolated from other rooms and acoustic distractions did not occur.

Testing Procedure

On the day of testing, the rats were brought to the testing room and left for a 3-h period to acclimatize to the novel surroundings. They had continual access to food and water during this period and remained with their housing companions. Each rat was injected IP with a single dose of SCH23390 (0.0, 0.10, or 0.25 mg/kg/ml; n = 8/group), or raclopride (0.0, 0.05, or 0.10 mg/kg/ml; n = 10/group) 20 min prior to testing by an investigator using coded drug bottles. In preliminary investigations we noted that doses above these produced locomotor retardation, and we excluded them from further study. Individual rats were placed into the middle of the white compartment at the start of the trial and left for 5 min. The group order was counterbalanced according to a Latin square design. At the conclusion of the test period, the rats were returned to their cages, and another animal was placed into the box. In between rats, the box was cleaned out with a 70% alcohol solution.

Behavioral Measures

The videotapes made on the day of testing were later scored by an investigator who was blind to the pretreatment regime. Each animal was scored for the following measures: 1) time to exit from the white compartment to the black; 2) time to reenter the white compartment from the black; 3) total time in the white compartment; 4) activity (squares crossed per unit time) in the white compartment; 5) activity (squares crossed per unit time) in the black compartment; and 6) number of crossings between the black and white chambers. To control for changes in activity levels due to differences in time distribution (i.e., rats spending more time in the black chamber invariably cross more squares), measures of activity are expressed as squares crossed per 10 s. These behavioral measures were selected because they had previously been seen to provide a reliable measure of anxiety (30–33).

Drugs

SCH23390 (Schering Corporation, Bloomfield, NJ) and raclopride (RBI, UK) D_1- and D_2-antagonists, respectively, at the above concentrations were used. These were prepared fresh each day in distilled water.

Statistical Analysis

All dependent measures were assessed using univariate analyses of variance (ANOVA). Each drug was analyzed independently from the other. Post hoc tests were performed using Student's *t*-test, applying a Bonferroni correction procedure to maintain the pairwise comparison alpha level at 0.05 and minimize type 1 errors (4).

RESULTS

Time to Exit From the White Chamber

There was no effect of SCH23390 on this variable, $F(2, 21) = 0.14$, NS, but ANOVA did reveal a significant effect of raclopride, $F(2, 27) = 6.1$, $p < 0.01$. As shown in Fig. 1, both doses of raclopride significantly reduced exit latencies ($ps < 0.01$) compared to the VEH group.

Time to Reenter White Chamber

Analysis of these data again failed to isolate any effect of SCH23390 administration with $F(2, 21) = 0.07$, NS. Raclopride, on the other hand, did affect this measure as demonstrated by ANOVA with $F(2, 27) = 4.6$, $p < 0.03$. Both the 0.05 and 0.10 doses of raclopride significantly delayed reentry into the white compartment compared to the VEH group ($ps <$ 0.04 and 0.03, respectively). These data are also depicted in Fig. 1. The contrasting effects of raclopride on times to exit and reenter the white chamber argue against any motoric deficits underlying these effects.

Time Spent in White Chamber

ANOVA on data from SCH23390-treated rats revealed no significant effect, $F(2, 21) = 0.14$, NS, but there was a significant effect of raclopride administration, $F(2, 27) = 5.5$, $p <$ 0.01. Raclopride, in a dose-dependent fashion, decreased total time spent in the white compartment ($ps < 0.01$–0.04). These data are illustrated in Fig. 1.

Number of Intercompartmental Crossings

Neither SCH23390 nor raclopride had any obvious effect on crossing behavior with $F(2, 21) = 0.16$, NS, and $F(2, 27) =$ 1.7, NS, respectively. These data are shown in Fig. 2.

Locomotor Activity in Black and White Chambers

Figure 2 also shows results from analysis of activity measures. Raclopride, but not SCH23390, affected activity scores in the white compartment, $F(2, 27) = 4.2$, $p < 0.03$. Post hoc comaprisons revealed that the 0.05 dose of raclopride significantly increased locomotor activity ($p < 0.01$) compared to the VEH group. Neither drug affected activity measures in the black compartment, however.

DISCUSSION

The results of the present study confirm that DA systems are involved in modulating anxiety and specifically indicate that D_2, but not D_1, receptors are central to the result. It is intriguing that raclopride produced enhanced anxiety in the black-white box because the effect can occur at postsynaptic receptors, thus implicating decreased DA activity as a mecha-

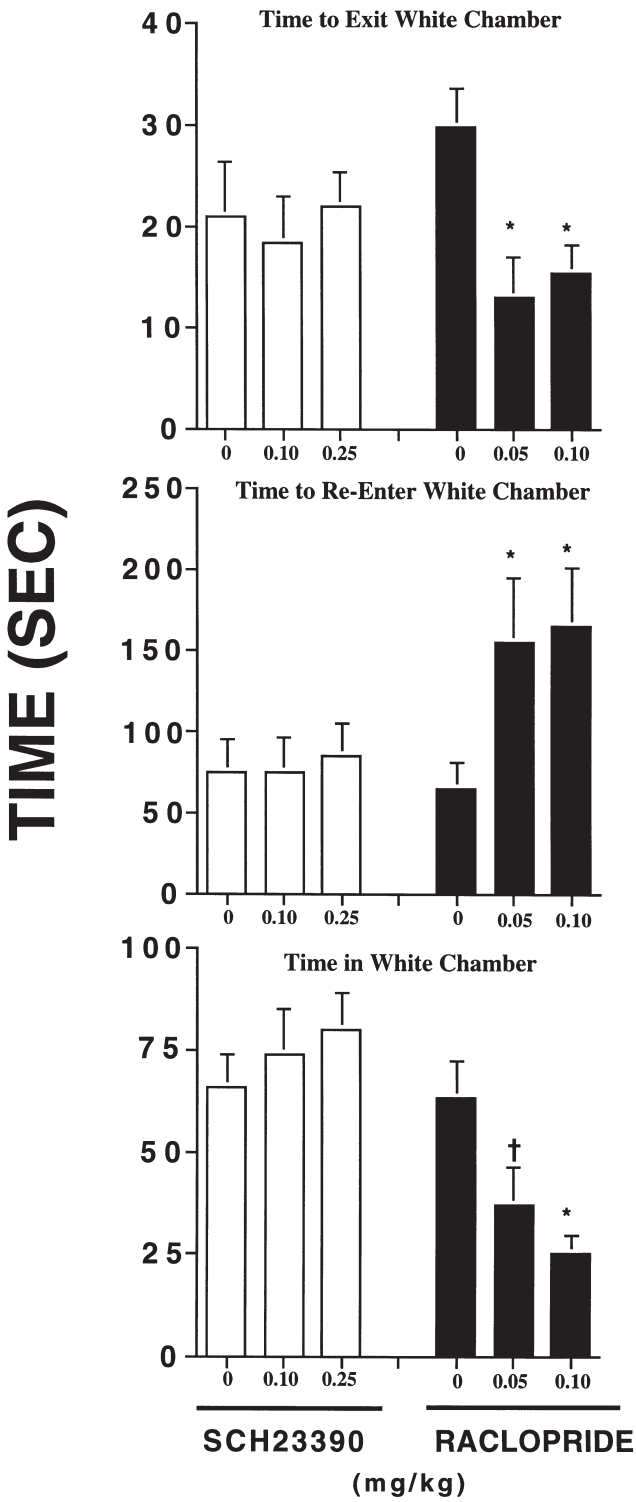

FIG. 1. Latencies to exit from the white chamber to the black chamber (top panel) following initial placement in the black-white box. Middle panel shows reentry times to the white from the black chamber. Bottom graph shows total time spent in the white chamber for the entire test trial. Values shown are means ± SEM. *Significantly different from VEH control group ($p < 0.01$); †significantly different from VEH control group ($p < 0.05$).

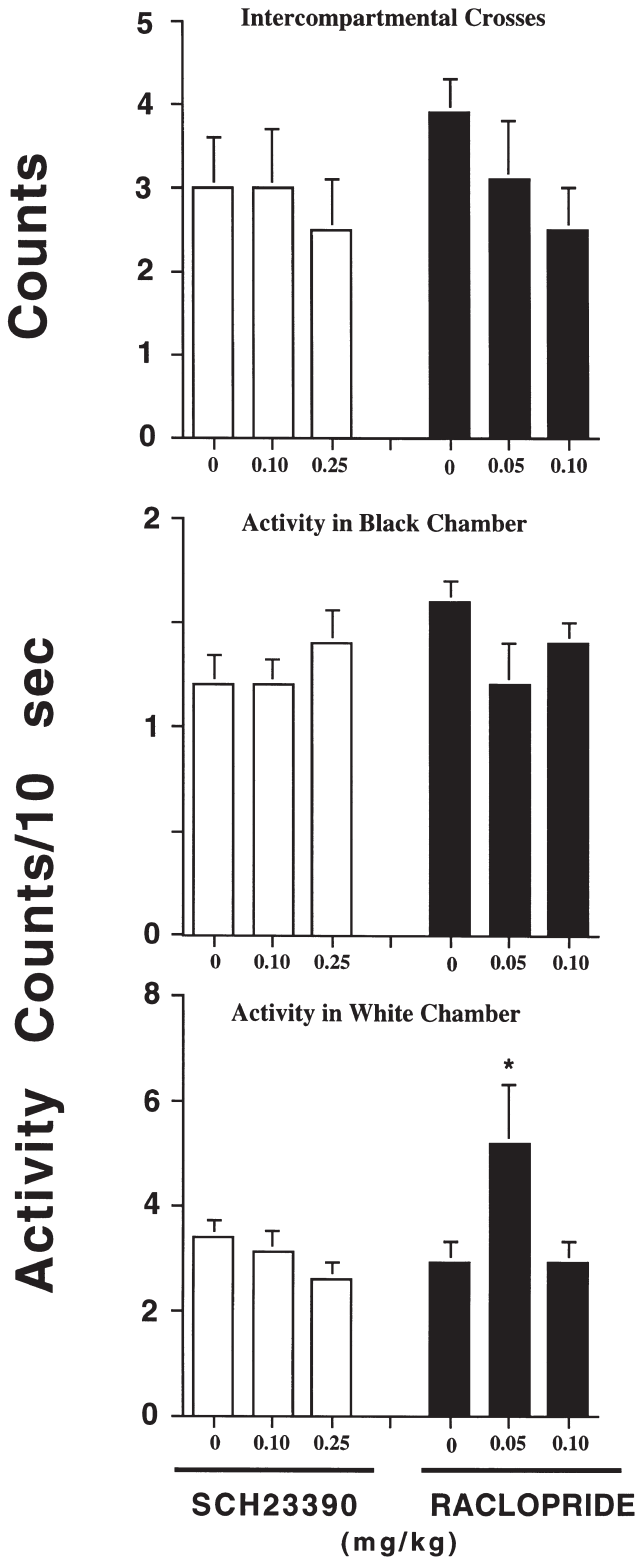

FIG. 2. Graphs showing locomotor activity for all groups. Top panel shows overall intercompartmental crossing behavior. Middle panel shows activity rates in the black compartment, and the bottom panel depicts activity rates in the white chamber. Values shown are means ±SEM. *Significantly different from VEH-treated rats ($p < 0.05$).

nism of anxiety. Alternatively, raclopride can stimulate somatodendritic autoreceptors that regulate DA release (15); blocking these sites would increase DA availability at postsynaptic receptors. In fact, Simon et al. (29) argued that D_2-mediated anxiogenesis was actually due to enhanced DA release because the D_1 antagonist SCH23390 blocked the effect. These contentions are speculative, however. If it were the case that enhanced D_1 activity was anxiogenic it would seem probable that SCH23390 should modulate intrinsic levels of anxiety induced by placing rats in the black-white box. However, neither Simon et al.'s (29) nor our present results showed any effect of SCH23390 on its own. Presumably, this means that basal DA activity has little significance for anxiety, but is relevant when levels are augmented (by autoreceptor blockade, or by postsynaptic DA receptor agonists). Moreover, there were no obvious motor effects that could account for or interfere with the measures of anxiety reported here. At the doses tested here, raclopride produced an increase in activity, but only in the white compartment. If a paucity of movement was hypothesized to influence measures of anxiety, then times to exit and reenter the white compartment would be similar. Thus, a change in anxiety is reflected by these times being opposite in direction, which is the effect we observed.

In previous studies, we reported that cholinergic blockade increased anxiety in the black-white box (30). We have also seen that cholinergic blockade aimed specifically at the hippocampus can increase the amount of anxiety expressed by rats, although qualitatively different from that resulting from systemic cholinergic blockade (32). There is substantial evidence to support the contention that the hippocampus is an important regulator of behavioral and physiological measures of arousal (9,17,18). Recent studies have demonstrated that DA systems regulate hippocampal cholinergic function. For instance, Day and Fibiger (8) have shown that *d*-amphetamine–stimulated release of acetylcholine (ACh) in vivo is blocked by D_1, but not D_2, receptors. Similarly, Hersi et al. (19) reported that the D_1 agonist SKF38393 elicited ACh release in the hippocampus, although SCH23390 had no intrinsic effect on its own. The lack of an effect of D_2 agonists or antagonists on ACh release would argue against the notion that raclopride produces anxiogenesis via an action at hippocampal cholinergic terminals. Presumably, this means that mesocortical or mesoaccumbens DA projections are likely targeted by raclopride to affect anxiety. Although it appears highly improbable that the hippocampus is involved directly in the effects of DA agents on anxiety, O'Donnell and Grace (24) have suggested that mesoaccumbens neurons can only be brought to depolarization if hippocampal–cortical afferents are intact and active. They argue that the hippocampus gates PFC inputs to the NAcc. Thus anxiety may be controlled by numerous foci that form a neural network involving both DA projections to NAcc and hippocampal cholinergic systems.

Stress-dependent induction of DA activity, and the corresponding augmentation of anxiety, is both surprising and intriguing because of the wealth of evidence for DA projections in the mediation of reward and motivated behaviors (16,25,35). It seems paradoxical that both aversive and rewarding stimuli would have similar neurochemical substrates. It may be argued that this commonality reflects a dynamic balance between pleasurable and unpleasurable environmental influences; what is perceived as rewarding and pleasant is contrasted with simultaneous perceptions of negative events, and both are translated into meso-NAcc/PFC DA activation. Alternatively, DA neuronal activity may represent some basic atten-

tional mechanism necessary for producing motivated behaviors (27).

In conclusion, we have demonstrated that D_2 receptor blockade enhances anxiety in rats. Although considerable evidence suggests that the hippocampus is important for the regulation of anxiety, it is likely that mesoaccumbens or mesocortical DA projections are involved in the DA effect on anxiety. It remains possible that hippocampal–PFC–NAcc afferents form part of a neural network controlling the expression of anxiety.

ACKNOWLEDGEMENTS

This work was supported by the Costall-Naylor research fund, and an establishment grant from the University of Bradford to J.W.S. The authors thank Susan J. McLaughlin for editorial assistance.

REFERENCES

1. Abercrombie, E. D.; Keefe, K. A.; DiFrischia, D. S.; Zigmond, M. J.: Differential effects of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial prefrontal cortex. J. Neurochem. 52:1655–1658; 1989.
2. Andreatini, R.; Leite, J. R.: Evidence against the involvement of ACTH/CRF release or corticosteroid receptors in the anxiolytic effect of corticosterone. Braz. J. Med. Biol. Res. 27:1237–1241; 1994.
3. Bradbury, M. J.; Akana, S. F.; Cascio, C. S.; Levin, N.; Jacobson, L.; Dallman, M. F.: Regulation of basal ACTH secretion by corticosterone is mediated by both type I (MR) and type II (GR) receptors in rat brain. J. Steroid Biochem. Mol. Biol. 40:133–142; 1991.
4. Bray, J. H.; Maxwell, S. E.: Analyzing and interpreting significant MANOVAs. J. Educat. Res. 52:340–367; 1982.
5. Cenci, M. A.; Kalen, P.; Mandel, R. J.; Bjorklund, A.: Regional differences in the regulation of dopamine and noradrenaline release in medial frontal cortex, nucleus accumbens and caudate-putamen: A microdialysis study in the rat. Brain Res. 581:217–228; 1992.
6. Costall, B.; Domeney, A. M.; Kelly, E. M.; Tomkins, D. M.; Naylor, R. J.; Wong, E. H. F.; Smith, W. L.; Whiting, R. L.; Eglen, R. M.: The effects of the 5-HT_3 receptor antagonist, RS-42358-197, in animal models of anxiety. Eur. J. Pharmacol. 234:91–99; 1993.
7. Costall, B.; Jones, B. J.; Kelly, M. E.; Naylor, R. J.; Tomkins, D. M.: Exploration of mice in a black and white test box: Validation as a model of anxiety. Pharmacol. Biochem. Behav. 32:777–785; 1989.
8. Day, J. C.; Fibiger, H. C.: Dopaminergic regulation of septohippocampal cholinergic neurons. J. Neurochem. 63:2086–2092; 1994.
9. Delacour, J.: A central activation role for the hippocampus: A viewpoint. Neurosci. Res. Commun. 16:1–10; 1995.
10. Dunn, A. J.; File, S. E.: Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and neostriatum. Physiol. Behav. 31:511–513; 1983.
11. Feenstra, M. G. P.; Botterblom, M. H. A.; van Uum, J. F. M.: Novelty-induced increase in dopamine release in the rat prefrontal cortex in vivo: Inhibition by diazepam. Neurosci. Lett. 189:81–84; 1995.
12. Feldman, S.; Weidenfeld, J.: Neural mechanisms involved in the corticosteroid feedback effects on the hypothalomo–pituitary–adrenocortical axis. Prog. Neurobiol. 45:129–141; 1995.
13. File, S. E.; Velluci, S. V.; Wendlandt, S.: Corticosterone—An anxiogenic or an anxiolytic agent? J. Pharm. Pharmacol. 31:300–305; 1979.
14. Gilad, G. M.; Habey, J. M.; Gilad, V. H.: Presynaptic effects of glucocorticoids on dopaminergic and cholinergic synaptosomes. Implications for rapid endocrine-neuronal interactions in stress. Life Sci. 40:2401–2408; 1987.
15. Grace, A. A.; Bunney, B. S.: Induction of depolarization block in midbrain DA neurons by repeated administration of haloperidol: Analysis using in vivo intracellular recording. J. Pharmacol. Exp. Ther. 238:1092–1100; 1986.
16. Gratton, A.; Wise, R. A.: Drug- and behavior-associated changes in dopamine-related electrochemical signals during intravenous cocaine self-administration in rats. J. Neurosci. 14:4130–4146; 1994.
17. Gray, J. A.: The neuropsychology of anxiety. Oxford: Oxford University Press; 1982.
18. Hasselmo, M. E.: Neuromodulation and cortical function: Modelling the physiological basis of behavior. Behav. Brain Res. 67:1–27; 1995.
19. Hersi, A. I.; Richard, J. W.; Gaudrea, P.; Quirion, R.: Local modulation of hippocampal acetylcholine release by dopamine D_1 receptors: A combined receptor autoradiagraphy and in vivo dialysis study. J. Neurosci. 15:7150–7157; 1995.
20. Imperato, A.; Puglisi-Allegra, S.; Casolini, P.; Zocchi, A.; Angelucci, L.: Stress-induced enhancement of dopamine and acetylcholine release in limbic structures: Role of corticosterone. Eur. J. Pharmacol. 165:337–338; 1989.
21. Lavielle, S.; Tassin, J. P.; Thierry, A. M.; Blanc, G.; Herve, D.; Barthelemy, C.; Glowinski, J.: Blockade by benzodiazepines of the selective high increase in dopamine turnover induced by stress in mesocortical dopamine neurons in the rat. Brain Res. 168:585–594; 1978.
22. Meaney, M. J.; O'Donnell, D.; Viau, V.; Bhatnagar, S.; Sarrieau, A.; Smythe, J. W.; Shanks, N.; Walker, C. D.: Corticosteroid receptors in the rat brain and pituitary during development and hypothalamic–pituitary–adrenal (HPA) function. In: McLaughlin, P.; Zagon, I., eds. Receptors and the developing nervous system. London: Chapman and Hill; 1993:163–201.
23. Murphy, D.; Costall, B.; Smythe, J. W.: Yohimbine's anxiogenic effect in the black-white box can be reduced by chlordiazepoxide: confirmation of test validity for rats. Br. J. Pharmacol. 119:63P; 1996.
24. O'Donnell, P.; Grace, A. A.: Synaptic interactions among excitatory afferents to nucleus accumbens neurons: Hippocampal gating of prefrontal cortical input. J. Neurosci. 15:3622–-3639; 1995.
25. Roberts, D. C. S.; Koob, G. F.; Klonoff, P.; Fibiger, H. C.: Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. 12:781–787; 1980.
26. Roth, R. H.; Tam, S. Y.; Ida, Y.; Yang, J. X.; Deutch, A. Y.: Stress and the mesocorticolimbic dopamine systems. Ann. NY Acad. Sci. 537:138–147; 1988.
27. Schultz, W.; Apicella, P.; Ljungberg, T.: Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. J. Neurosci. 13:900–913; 1993.
28. Simon, P.; Panissaud, C.; Costentin, J.: Anxiogenic-like effects induced by stimulation of dopamine receptors. Pharmacol. Biochem. Behav. 45:685–690; 1993.
29. Simon, P.; Panissaud, C.; Costentin, J.: Sulpiride anxiogenic-like effect inhibition by a D_1 dopamine receptor antagonist. Neuroreport 3:941–942; 1992.
30. Smythe, J. W.; Murphy, D.; Bhatnagar, S.; Timothy, C.; Costall, B.: Muscarinic antagonists produce anxiogenesis in rats tested in the Black-white box. Pharmacol. Biochem. Behav. 54:57–63; 1996.
31. Smythe, J. W.; Murphy, D.; Timothy, C.; Costall, B.: Hippocampal mineralocorticoid, but not glucocorticoid, receptors modulate anxiety-like behavior (ALB) as assessed by the black-white box. Pharmacol. Biochem. Behav. 56:507–513; 1997.
32. Smythe, J. W.; Bhatnagar, S.; Murphy, D.; Timothy, C.; Costall, B.: The effects of intrahippocampal scopolamine infusions on anxiety in rats as measured by the black-white box. Brain Res. Bull. 45:89–93; 1998.
33. Smythe, J. W.; Murphy, D.; Timothy, C.; Costall, B.: Exogenous corticosterone is anxiogenic in rats tested in the black-white box. Br. J. Pharmacol. 118:62P; 1995.
34. Thierry, A. M.; Tassin, J. P.; Blanc, G.; Glowinski, J.: Selective activation of the mesocortical dopaminergic system by stress. Nature 263:242–244; 1976.
35. Wise, R. A.: Catecholamine theories of reward: A critical review. Brain Res. 152:215–247; 1978.