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Possible Dopaminergic Stimulation of Locus Coeruleus α_1 -Adrenoceptors Involved in Behavioral Activation

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Abstract

α_1 -Adrenoceptors of the locus coeruleus (LC) have been implicated in behavioral activation in novel surroundings, but the endogenous agonist that activates these receptors has not been established. In addition to the canonical activation of α_1 -receptors by norepinephrine (NE), there is evidence that dopamine (DA) may also activate certain brain α_1 -receptors. This study examined the contribution of DA to exploratory activity in a novel cage by determining the effect of infusion of various dopaminergic and adrenergic drugs into the mouse LC. It was found that the D2/D3 agonist, quinpirole, which selectively blocks the release of CNS DA, produced a dose-dependent and virtually complete abolition of exploration and all movement in the novel cage test. The quinpirole-induced inactivity was significantly attenuated by coinfusion of DA but not by the D1 agonist, SKF38390. Furthermore, the DA attenuation of quinpirole inactivity was blocked by coinfusion of the α_1 -adrenergic receptor antagonist, terazosin, but not by the D1 receptor antagonist, SCH23390. LC infusions of either quinpirole or terazosin also produced profound inactivity in DA- β -hydroxylase knockout (Dbh $-/-$) mice that lack NE, indicating that their behavioral effects were not due to an alteration of the release or action of LC NE. Measurement of endogenous DA, NE, and 5HT and their metabolites in the LC during exposure to the novel cage indicated an increase in the turnover of DA and NE but not 5HT. These results indicate that DA is a candidate as an endogenous agonist for behaviorally activating LC α_1 -receptors and may play a role in the activation of this nucleus by novel surroundings.

Keywords

locus coeruleus; α_1 -adrenoceptor; DA; motor activity; novelty

INTRODUCTION

α_1 -Adrenoceptors in widely distributed regions of the CNS have been shown to play a significant role in behavioral activation under a variety of conditions (Stone et al., 2007b). Pharmacological blockade of these receptors in the motor and piriform cortex, nucleus accumbens, preoptic area, lateral hypothalamus, vermis cerebellum, locus coeruleus (LC), and dorsal raphe produces immobility in a novel test cage, whereas stimulation leads to behavioral

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activation in the familiar home cage (Stone et al., 2004a,b). The LC appears to be a key region in this system in that it contains a dense concentration of α_1 -receptors (Jones et al., 1985), which when blocked with locally injected terazosin produces one of the strongest degrees of immobility and catalepsy in the novel cage test (7 out of 10 min immobile), and when stimulated with catecholamines produces vigorous exploratory behavior in familiar surroundings (Stone et al., 2004a,b). Blockade of LC α_1 -receptors also impairs lateral hypothalamic self-stimulation behavior in rats (Lin et al., 2007), suggesting a broad role in positively motivated behaviors. Presumably, LC activation via stimulation of local α_1 -receptors produces increased postsynaptic α_1 -noradrenergic neurotransmission in one or more of these target areas and is responsible for behavioral activation under these conditions.

The endogenous neurotransmitter that activates LC α_1 -receptors is either norepinephrine (NE), dopamine (DA), or epinephrine (EPI). EPI-containing nerve terminals emanating from the paragigantocellularis nucleus of the ventrolateral medulla are known to innervate the LC (Astier et al., 1990). Although we have shown that exogenous EPI injected in the LC can stimulate behavioral activity in the home cage, only a portion of this effect is blocked by an α_1 -receptor antagonist (Stone et al., 2003). Furthermore, preliminary experiments in our laboratory have failed to reveal substantial amounts of EPI in homogenates of the LC or reliable inhibition of behavioral activity after local inhibition of EPI biosynthesis in the LC by the phenylethanolamine-*N*-methyltransferase (PNMT) inhibitor dichloromethyl-benzylamine (Stone, Quartermain, Lin, unpublished results).

There is more consistent evidence that either or both NE and DA are the endogenous agonists for these receptors. With respect to NE, the high concentrations of extracellular NE found in the LC originates from either recurrent noradrenergic axon collaterals (Nakamura et al., 1988) or somatodendritic release from LC noradrenergic neurons (Fernández-Pastor et al., 2005; Singewald and Philippu, 1998). We and others (De Sarro et al., 1987; Stone et al., 2005) have shown that local reduction of NE release in the LC by injection of α_2 -adrenoceptor agonists produces marked immobility in the novel cage test in mice and rats.

With respect to DA, although tract tracing and immunohistochemical studies have been inconclusive regarding a dopaminergic innervation of the LC (Deutch et al., 1986; Somerville et al., 2007), substantial amounts of this catecholamine (Dishman et al., 1997; Versteeg et al., 1976) and high concentrations of its metabolite, dihydroxyphenylacetic acid, are found in this nucleus (Lambas-Senas et al., 1990), although its cellular localization is still unclear. Although a microdialysis study of the cat LC found relatively small amounts of extracellular DA (Crochet and Sakai, 1999), a push-pull cannula study in the same species found equal levels of extracellular DA and NE in this nucleus (Kaehler et al., 1999), which is not unexpected given that DA is the immediate precursor of NE. In addition, an early study showed that activation of the DAergic cell bodies in the ventral tegmental area produced activation of LC neurons as evidenced by the accumulation of metabolites of NE in noradrenergic projection areas (Deutch et al., 1986). Although DA possesses only 1/50 the affinity of NE at α_1 -receptors (Leedham and Pennefather, 1986), endogenous DA has been shown conclusively by electrophysiological methods to activate these receptors in the avian preoptic area (Cornil et al., 2002) and is also known to be equally efficacious as NE at these receptors in cell culture (Rey et al., 2001; Zhang et al., 2004).

In addition, the arousing effect of the stimulant modafinil, which is dependent on α_1 -receptor activity (Duteil et al., 1990; Stone et al., 2002), has been found to be potently inhibited by selective blockade of DA release with the D2 dopaminergic agonist, quinpirole (Wisor and Eriksson, 2005). The latter authors have suggested that DA is an endogenous agonist at α_1 -receptors involved in arousal.

A contribution of DA to the activation of the LC would have significant implications for an understanding of the role of this nucleus in behavioral regulation and motivation. Therefore, the present experiments were undertaken to test this hypothesis. This was accomplished by examining the effect of microinjection of various selective dopaminergic and adrenergic agents in the vicinity of the mouse LC on novel cage-induced behavioral activity in both outbred and genetically altered (NE-deficient) mice.

MATERIALS AND METHODS

Subjects

Swiss Webster male mice (Taconic), 8–10 weeks old, female DA- β -hydroxylase knockout (Dbh $-/-$) mice that completely lack NE, and female and male wild-type control mice, 4–9 months of age, of a mixed C57BL/6/J and 129SvEv genetic background (Thomas et al., 1998) were subjects. The animals were housed singly with nesting material for 5 days prior to surgery in standard polycarbonate mouse cages (12.5 cm \times 17 cm \times 28 cm) at a room temperature of 22°C \pm 1°C under a 12 h light/dark cycle (lights on 05:00 h). Food and water were available ad libitum.

Surgery

Mice were anesthetized with pentobarbital (70 mg/kg) and implanted stereotaxically with unilateral 26 ga cannula guide tubes in the dorsal pons in the region of the LC at the following coordinates (–5.5 mm posterior to Bregma, \pm 0.9 mm lateral to the mid-line, 3.9 mm beneath the surface of the skull). We have shown in a previous study that unilateral blockade of LC α_1 -adrenoceptors in the mouse is sufficient to produce maximal inactivity in the 10-min novel cage test (Stone et al., 2004a,b). All animals were given 10 days for recovery prior to behavioral testing.

Procedure

All experiments were performed between 1000 and 1400 h. Mice were gently restrained under a double layer of gauze on a corkboard and a 33 ga cannula connected by PE tubing to a syringe pump (Razel) was inserted into the cannula guide tube (protruding 1 mm below the bottom of the guide). A total of 350 nl of solution was infused at 150 nl/min over a 2.3-min period with the cannula remaining in place for 30 s after infusion. The animals received either vehicle (saline), the D2 dopaminergic agonist, quinpirole (Q), DA, the D1/D5 agonist, SKF38393 (SKF), the α_1 -adrenoceptor antagonist, terazosin (T), or the D1/D5 antagonist, SCH23390 (SCH), singly or in combination, in doses ranging from 0.3 to 30 nmol per mouse. All drugs were prepared fresh each day in saline. Minimum doses were determined empirically in pilot studies. Immediately following the injection, the animal was transferred to a clean unfamiliar mouse cage of identical type and dimensions as the home cage (“novel home cage”) with Bed-O-Cob bedding (0.63 mm) and was videotaped for the next 10 min. The animals received four such injections at 4 days intervals with the first and fourth using Q at 3 nmol, and the second and third being any of the drugs singly or in combination. Thus different groups of animals received two different drug treatments in addition to the Q3 trials. A given drug treatment was represented equally on the second and third injections.

Behavioral ratings

Videotapes were rated either by a trained observer (in blind fashion) for a measure of total behavioral activity [the number of gross movements (GM) made] and time immobile (no observable movements) or by a videotracking system (Smart System, San Diego Inst) for ambulation (the number of times the animal crossed the long and short axes of the cage). GMs were defined as any movement involving at least the head plus forelimbs that was followed by

a momentary pause. These include ambulations to a cage wall and other discrete forward movements, rearing responses, stretch and attend responses, turns and large grooming movements. Interrater agreement on this measure has averaged above $r = 0.9$ in this laboratory. Central $\alpha 1$ -adrenoceptor activity has been shown in previous studies to have a general modulating effect on a variety of different active behaviors, and the number of GM made in the novel cage test has been shown to be a function of this activity (Stone et al., 1999, 2001, 2004a,b). All experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (1996), and were approved by the New York University School of Medicine IUCAC.

DA turnover in the vicinity of the LC

Undisturbed mice or mice exposed to a novel home cage for 20 min were sacrificed by rapid decapitation. The LC and adjacent tissue were grossly dissected over ice as a $1.5 \text{ mm} \times 1.5 \text{ mm} \times 1 \text{ mm}$ block of tissue centering on the lateral extent of IV. ventricle from a 1-mm brain section between -5 and -6 mm Bregma. NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), DA, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were assayed by high-performance liquid chromatography (HPLC) as described by Dunn (1993).

Histology

After the experiment, the implanted animals were deeply anesthetized and injected through the cannula with 350 μl of 2% methyl blue. Brains were fixed in 10% formalin for 3 days and then sectioned for determination of cannula location.

Statistical analysis

The individual data points comprised 2–3 observations per animal taken under different drug conditions. All analyses involved either one- or two-way ANOVAs. Because of the diverse nature of drug treatments used there were no significant correlations between responses to the different drug treatments across the four trials. Therefore, each of the data points was treated as an independent observation. All individual mean comparisons were planned and were Bonferroni-corrected.

RESULTS

Localization of quinpirole effect

An initial group of 44 animals that were implanted at various sites in the pons and cerebellum were injected with Q at 3 nmol and assessed for behavioral activity in the novel home cage test. Of these, 19 (43.2%) developed immobility of greater than 7.5 min out of the 10 min test and were judged as Q3-positive animals. Histological analysis (Fig. 1) indicated that of the 19, 4 were located in the 4th ventricle and were excluded, whereas 14 of the remaining 15 (93.3%) had cannulas within 0.5 mm of the border of the LC core, and only 9 (31.0%) of the 25 Q3-negative animals had cannulas this close. This difference in percentage was highly significant by Fisher's exact test ($P < 0.0001$).

Alternately, if the group of 40 mice was divided into those having cannulas within 0.5 mm of the LC and those outside this distance, a similar result was obtained, with the "within" group having more than a threefold higher level of immobility than the "outside" group (within, 6.73 ± 0.64 min (23); outside, 2.21 ± 0.56 min (17), $t_{38} = 5.24$, $P < 0.001$).

In subsequent experiments, we therefore adopted the procedure of first screening all implanted animals with an initial Q3 test and only using Q3-positive animals for two subsequent drug challenges at 4 days intervals (the location of the novel home cage in the laboratory was

changed for each test). Pilot experiments showed that the majority of animals had reliable responses to repeated administrations of this drug for up to four injections at 4 days intervals. A final Q3 test (fourth injection) was therefore employed to determine if the initial response to the drug was maintained throughout the series of injections. Of a total of 116 initially Q3-positive animals, 62.2% maintained the immobility response to the drug on the 4th challenge, and of these, 75.3% were found to have cannulas within 0.5 mm of the LC and were used in the final analysis. All responses to Q3 were computed as the average of the first and fourth injection, whereas all responses to other Q doses and to all other drugs were based on responses to the second or third injections. There was a high and significant correlation between immobility scores of separate groups of mice given the same drug treatments on different tests ($r_{10} = 0.92$, $P < 0.001$), which suggests that responses to the same treatments were stable and largely independent of order effects.

Dose response to quinpirole and comparison with terazosin and SCH23390

The effect of Q at doses between 0.3 and 3 nmol on activity level in the novel cage is shown in Figure 2. A one-way ANOVA comprising the vehicle and all Q doses indicated that the drug produced significant dose-dependent reductions of both GM ($F_{3,27} = 91.23$, $P < 0.0001$) and ambulation ($F_{3,27} = 25.98$, $P < 0.0001$) and a significant dose-dependent increase in immobility ($F_{3,27} = 73.57$, $P < 0.0001$). Figure 2 also shows the effects of terazosin 3 nmol (T3) and SCH23390 3 nmol (SCH3). Comparison of the vehicle, Q3, T3, and SCH3 conditions by one-way ANOVAs followed by posthoc Bonferroni tests revealed that T3 significantly reduced GM ($P < 0.0001$) and ambulation ($P < 0.0001$) and increased immobility ($P < 0.0001$), and that SCH3 also reduced GM ($P < 0.01$) and ambulation ($P < 0.005$), but produced only a borderline increase in immobility ($P < 0.06$). However, the degree of inactivity produced by Q3 on all behavioral measures was significantly greater than that produced by SCH3 (all P s < 0.001), whereas there were no significant differences between the Q3 and T3 conditions on any measure.

Attenuation of Q3 immobility by DA but not by SKF38393

The effect of coinfusion of DA and SKF38393 (SKF) at 10 and 30 nmol on Q3-induced inactivity is shown in Figure 3. A one-way ANOVA comprising only the DA conditions (0, 10, and 30 nmol) showed that the catecholamine produced a dose-dependent attenuation of the reduction in GM ($F_{2,24} = 14.98$, $P < 0.0001$) and ambulation ($F_{2,24} = 3.52$, $P < 0.05$), and of the increase in immobility ($F_{2,24} = 16.21$, $P < 0.0001$) caused by the D2 agonist (Fig. 3). In the absence of Q3, DA had no significant effect on any behavior at either dose (data not shown). The same analysis for the SKF conditions (0, 10, and 30 nmol) failed to reveal a significant action on Q3-induced immobility.

Blockade of the attenuating effect of DA by an antagonist of α_1 -adrenergic but not D1 dopaminergic receptors

The effects of T and SCH (both at 3 and 10 nmol) on the attenuating effect of DA (30 nmol) of Q3-induced inactivity are shown in Figure 4. In this figure, the Q3-DA30 group is the same as used in the foregoing comparison (3). A one-way ANOVA comprising the Q3-DA30 condition in the presence of 0, 3, and 10 nmol T showed that the α_1 -antagonist produced a blockade of the DA reversal effect that was significant for GM ($F_{2,27} = 8.63$, $P < 0.005$) and immobility ($F_{2,27} = 6.86$, $P = 0.005$), and was of borderline significance for ambulation ($F_{2,27} = 3.24$, $P < 0.06$). Analyses of linear trend components suggested that these effects were dose-dependent for GM ($F_{1,27} = 11.22$, $P < 0.005$) and for time immobile ($F_{1,27} = 9.08$, $P < 0.01$). The one-way ANOVA for SCH at 0, 3, and 10 nmol did not reveal a significant effect on any measure. Neither T nor SCH at 10 nmol significantly altered any of the behaviors of animals treated with only Q3 (data not shown).

Effect of quinpirole and terazosin in NE-deficient mice

Because of the smaller size and increased fragility of the *Dbh*^{-/-} mice, the initial screening injection was omitted and the animals were injected only three times with Q3, T3, and vehicle in that order. Eight of the 10 WT and 6 of the 11 *Dbh*^{-/-} mice were subsequently found to have cannulas located within 0.5 mm of the LC and completed the Q3 and T3 injections. Because of the loss of cannulas, only five of each group completed the vehicle injection. The results are shown in Figure 5. A 2 × 3 (genotype × drug) ANOVA revealed significant main effects of drug for each dependent measure (GM, $F_{2,31} = 13.08$, $P < 0.001$; ambulation, $F_{2,31} = 13.48$, $P < 0.001$; time immobile, $F_{2,31} = 16.19$, $P < 0.0001$), but there was no significant effects of genotype (GM, $F_{1,31} = 0.02$, NS; ambulation, $F_{1,31} = 2.15$, NS; time immobile, $F_{1,31} = 0.04$, NS) or interactions of genotype and drug for any dependent measure (GM, $F_{2,31} = 0.17$, NS; ambulation, $F_{2,31} = 1.67$, NS; time immobile, $F_{2,31} = 0.16$, NS).

Turnover of LC DA during exposure to novel home cage

The effects of novel home cage exposure on levels and turnover of DA, NE, and 5HT in the vicinity of the LC are shown in Table I. Novel cage exposure produced a highly significant increase in the concentration of DOPAC and in the ratios of DOPAC/DA, and MHPG/NE indicative of increases in the turnover of DA and NE. The concentrations of 5HT and 5HIAA and the 5HIAA/5HT ratio were not significantly altered.

DISCUSSION

These results provide indirect pharmacological evidence that DA can stimulate the population of α_1 -adrenoceptors in the vicinity of the LC that functions in behavioral activation in the novel home cage test. Quinpirole, a D2/D3 agonist that selectively reduces the release of CNS DA (Devoto et al., 2002), produced a significant and near total dose-dependent abolition of all movement in this test when injected within 0.5 mm of this nucleus, but was much less effective at greater distances. No loss of the righting reflex (maintenance of an upright position) was observed as would have occurred with a sedative effect. The quinpirole-induced immobility was significantly and dose-dependently reversed by the addition of DA, whereas DA given alone had no effect on nonquinpirole-treated animals who were already maximally active. The DA reversal appeared to be acting via stimulation of α_1 -adrenoceptors, since (a) it was significantly blocked by the α_1 -antagonist, terazosin; (b) it was not blocked by the D1 antagonist, SCH23390; and (c) the D1 agonist, SKF38390, failed to reverse the Q-induced inactivity. These data therefore add to the previous direct electrophysiological evidence of the stimulation of CNS α_1 -adrenoceptors by DA discussed earlier.

Since D2 receptors may be present on noradrenergic as well as dopaminergic neurons (Smiaowska and Legutko, 1992), it could be argued that the behavioral effects of quinpirole were due to an inhibition of NE release and a subsequent failure of NE to activate α_1 -receptors in the LC. This possibility, however, appears very unlikely because quinpirole and terazosin were equally effective in control and *Dbh*^{-/-} mice, which completely lack NE. Combining these results suggests that DA is an endogenous ligand for α_1 -receptors in the LC, although the source of the DA is uncertain. The DA could be coming from dopaminergic terminals that innervate the LC, or from the LC itself, which produces DA instead of NE in *Dbh*^{-/-} mice (Thomas et al., 1998). DA release from noradrenergic neurons has also been observed in wild-type animals. Because DA possesses only 1/50 the affinity of NE at α_1 -adrenoceptors, the question arises as to how this catecholamine can stimulate these receptors as suggested by the present and previous experiments. However, α_1 -receptors, are now known to form heterodimers that possess agonist properties different from individual subtypes (Hague et al., 2006). It is possible therefore that DA has a greater affinity for a receptor heterodimer than for individual α_1 -receptors. Since the only catecholamine present in the *Dbh*^{-/-} animals is DA, it is highly likely

that the latter compound is an agonist for some behaviorally activating α_1 -receptors in the NE-deficient mice. This is consistent with activation of α_1 - and α_2 -receptors in the avian preoptic area by endogenous DA discussed earlier. Whether this is also obtained in wild type mice is not presently clear, but it is known that both DA and NE are released at many sites in the CNS.

The presumed excitation of LC α_1 -receptors by DA must, of course, await further direct confirmation by appropriate electrophysiological methods. However, if DA is, in fact, responsible for the stimulation of these receptors during novel home cage exposure, it should be possible to demonstrate an increase in DA release near the LC during this stimulus. This was confirmed in the present experiment from measures of the ratio of DOPAC:DA in the dorsal pons in the vicinity of the LC of mice exposed to the novel home cage. It has been suggested that DOPAC in the LC arises from noradrenergic rather than dopaminergic neurons, because there is a high correlation between levels of this metabolite and the electrical activity of LC neurons (Buda et al., 1994). However, as discussed earlier, significant concentrations of DA have been demonstrated in the extracellular fluid near the LC, which makes it more likely that the metabolite arises from dopaminergic neurons. This is in agreement with the present finding that the level of DOPAC, a measure of turnover, was similar to the level of MHPG despite the low concentration of the parent amine DA. It is possible that the high correlation previously observed between LC activity and DOPAC concentrations may result from correlated activities between dopaminergic afferents and LC neurons. This is supported by the present finding of increased turnover of both DA and NE in the vicinity of the LC during novel cage exposure.

The exact location of the α_1 -receptors mediating behavioral activation in a novel environment also remains to be determined. Although radioligand binding experiments have detected the presence of α_1 -receptors in the LC (Chamba et al., 1991; Jones et al., 1985; Stone et al., 2004a,b), in situ hybridization techniques have failed to detect mRNA for α_1 -receptors in this region (Pieribone et al., 1994), suggesting that these receptors may be located on terminals from distal neurons (e.g., glutamatergic nerve terminals) that innervate the LC (Singewald and Philippu, 1998). One appealing hypothesis is that α_1 -receptors located on glutamatergic neurons innervating the LC increase glutamate release, which in turn increases the excitability of LC neurons. On the other hand, more recent in situ studies (Day et al., 1997) and more sensitive methods (i.e., RT-PCR) have detected α_1 -receptor mRNA expression by LC neurons (Osborne et al., 2002), and electrophysiological experiments indicate that activation of α_1 -receptors on LC dendrites increases excitability of these cells (Finlayson and Marshall, 1986; Ivanov and Aston-Jones, 1995; Nakamura et al., 1988). Interestingly, some excitatory effects of α_1 -receptor activation on LC neurons are most evident during early development but disappear in adults (Finlayson and Marshall, 1986; Nakamura et al., 1988), while others persist into adulthood (Osborne et al., 2002). We obtained our behavioral results in adult mice, while most of the aforementioned studies examined rats. Further investigation will be needed to determine the relative contribution of α_1 -receptors present on LC dendrites and those present on nerve terminals in the LC to the novelty-induced motor response.

The functional significance of the presumed dopaminergic excitation of LC α_1 -receptors is not presently understood. However, it may be related to the different types of behaviors mediated by this nucleus. The LC has long been known to be involved in both negative affect-related [stress (Weiss and Simson, 1985), opiate withdrawal (Taylor et al., 1988)] and positive affect-related behaviors [self stimulation (Lin et al., 2007) as well as reward-based operant behavior (Aston-Jones and Cohen, 2005)]. Novel home cage exploration is predominantly a positively motivated behavior in that it is accompanied by robust Fos expression in brain areas involved in approach behavior and motor/motivational processes but not in stress response regions (Stone et al., 2007a), and that animals will work to gain access to novel chambers (Klebaour and Bardo, 1999). Furthermore, DA is the primary monoamine neurotransmitter of brain

circuits mediating reward (Nestler and Carlezon, 2006). Therefore, it is possible that dopaminergic neurotransmission in the LC codes selectively for positive-affect related behaviors mediated by this nucleus.

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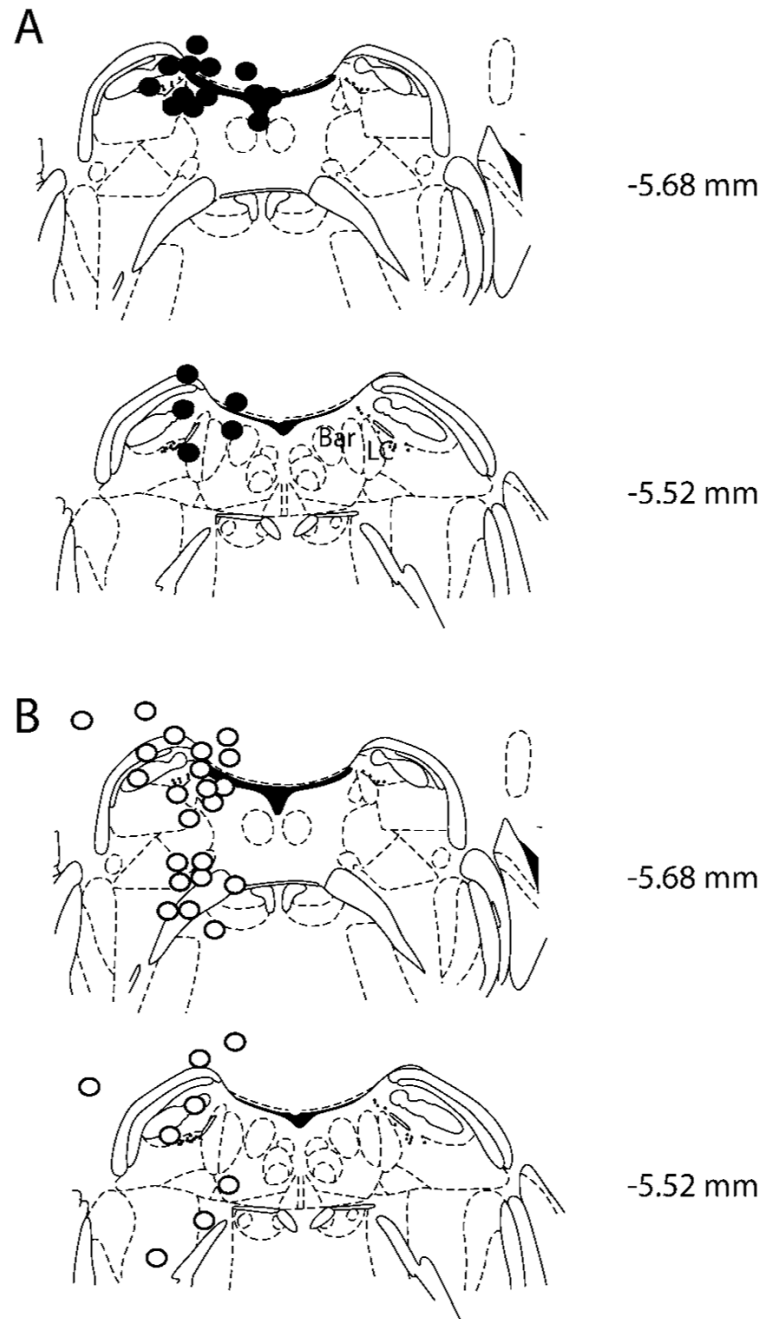


Fig. 1. Locations of cannula tips for quinpirole-positive (A) and -negative (B) animals in initial experiment. Quinpirole was infused at 3 nmol unilaterally. LC, locus coeruleus; BAR, Barrington's nucleus. Figures adapted from Franklin and Paxinos (1997).

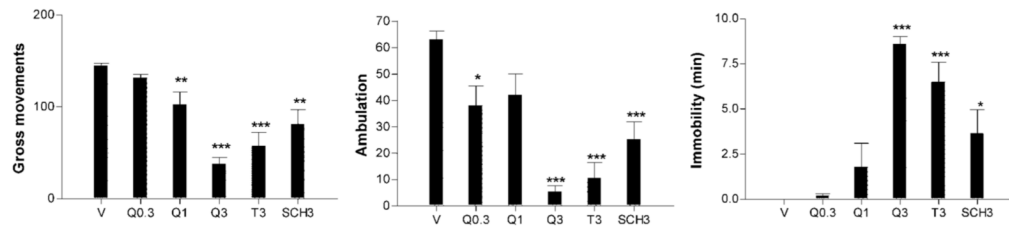


Fig. 2.

Effect of various doses of quinpirole (Q), terazosin (T), and SCH23390 (SCH) or vehicle (v, saline) in the LC on behavioral activation in novel home cage test. Values are means and SEMs of 7–10 determinations for Q 0.3–3 nmol and 5–7 determinations for T3 and SCH3. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. vehicle condition.

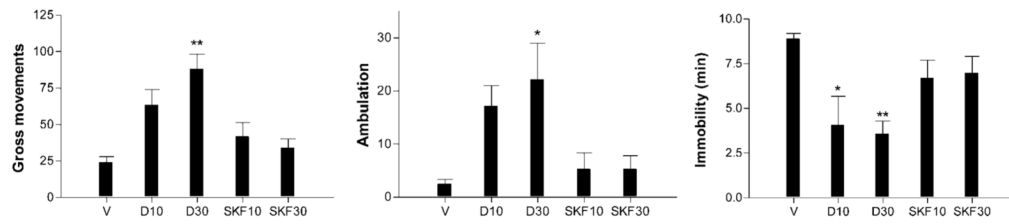


Fig. 3.

Dose-dependent reversal of quinpirole-induced inactivity by coinfusion of DA but not SKF38393. Mice were infused with quinpirole (3 nmol) in the presence of either DA or SKF at 0, 10, and 30 nmol. DA or SKF38393 were dissolved in the Q3-containing saline solution (veh). Means and SEMs of 6–10 determinations. * $P < 0.05$, ** $P < 0.001$ vs. vehicle.

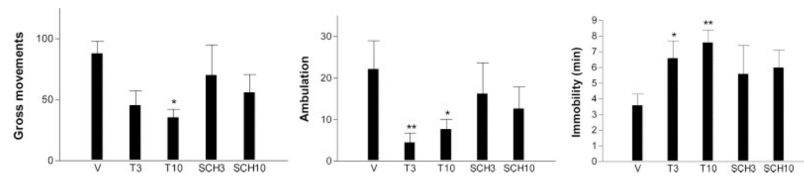


Fig. 4. Blockade of DA-reversal of quinpirole-inactivity by terazosin but not by SCH23390. Mice were infused with 3 nmol quinpirole + 30 nmol DA in the presence of either 0 (veh), 3 or 10 nmol of terazosin or SCH23390. Terazosin or SCH23390 were dissolved in the Q3-DA30-containing saline solution (veh). Means and SEMs of 6–10 determinations. * $P < 0.01$ vs. vehicle condition.

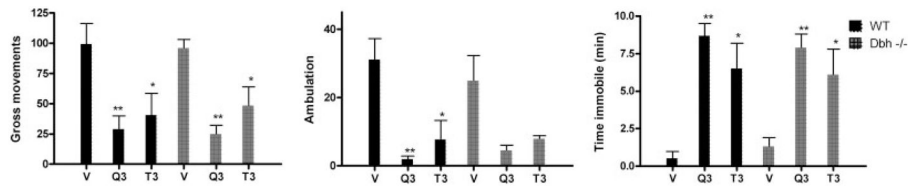


Fig. 5. Induction of inactivity in NE-deficient mice by quinpirole and terazosin. Dbh^{-/-} or wild type mice were infused with vehicle (v, saline), quinpirole or terazosin at 3 nmol. Means and SEMs of 6–8 determinations for Q and T and 5 determinations for vehicle. * $P < 0.05$, ** $P < 0.01$.

TABLE I
Effect of novel home cage exposure on turnover of NE, DA, and 5HT in the vicinity of the LC

| Group | DA | DOPAC | DOPAC/DA | NE | MHPG | MHPG/NE | 5HT | 5HIAA | 5HIAA/5HT |
|----------------|--------|--------|----------|-------|--------|---------|-------|-------|-----------|
| Control (9) | 0.019 | 0.015 | 0.786 | 0.349 | 0.016 | 0.047 | 0.398 | 0.485 | 1.109 |
| Novel cage (9) | 0.002 | 0.002 | 0.027 | 0.040 | 0.002 | 0.001 | 0.042 | 0.112 | 0.147 |
| | 0.023 | 0.025 | 1.049 | 0.369 | 0.023 | 0.060 | 0.406 | 0.568 | 1.281 |
| <i>t</i> | 0.001 | 0.003 | 0.072 | 0.032 | 0.003 | 0.003 | 0.038 | 0.134 | 0.189 |
| | -1.76 | -3.35 | 3.43 | -0.40 | 1.92 | 4.40 | -0.13 | -0.47 | -0.72 |
| <i>p</i> | 0.0960 | 0.0004 | 0.0034 | NS | 0.0720 | 0.0004 | NS | NS | NS |

Values are means and SEMs of groups of 9 mice. Values of amines are in ng/mg wet weight.