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Patrizia Nestby · Louk J.M.J. Vanderschuren
Taco J. De Vries · François Hogenboom
George Wardeh · Arie H. Mulder
Anton N.M. Schoffelmeer

Ethanol, like psychostimulants and morphine, causes long-lasting hyperreactivity of dopamine and acetylcholine neurons of rat nucleus accumbens: possible role in behavioural sensitization

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Abstract Repeated treatment of rats with ethanol (1 g/kg, once daily for 15 days) enhanced the locomotor effect of morphine, 3 weeks post-treatment. This ethanol-induced long-term behavioural sensitization to morphine was associated with an increase in the electrically evoked release of [^{3}H]dopamine (DA) and [^{14}C]acetylcholine (ACh) from nucleus accumbens slices. A similar enhanced responsiveness of accumbal dopaminergic and cholinergic neurons to depolarization was apparent 3 weeks after repeated morphine, amphetamine or cocaine administration. Prior ethanol exposure also caused a long-term enhancement of electrically evoked release of [^{3}H]DA and [^{14}C]ACh from slices of the caudate-putamen. Unlike the locomotor effect of morphine, that of amphetamine was not enhanced in ethanol-pretreated rats. These data indicate that ethanol administration may cause long-term behavioural sensitization associated with adaptive changes in dopaminergic and cholinergic neurons of rat nucleus accumbens and caudate-putamen. Furthermore, an enhanced reactivity of nucleus accumbens dopaminergic nerve terminals and dopamine-sensitive cholinergic neurons appears to be a common long-term neuroadaptive effect of distinct types of addictive drugs. However, since repeated ethanol exposure did not cause a long-term increase in the locomotor effect of amphetamine, these neuroadaptations may not always be sufficient to cause long-lasting behavioural (cross-)sensitization.

P. Nestby · L.J.M.J. Vanderschuren · T.J. De Vries
F. Hogenboom · G. Wardeh · A.H. Mulder
A.N.M. Schoffelmeer (✉)
Research Institute Neurosciences Vrije Universiteit,
Faculty of Medicine, Department of Pharmacology,
Free University, Van der Boechorststraat 7,
1081 BT Amsterdam, The Netherlands
Fax (+31) 20/4448100

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Introduction

Repeated opiate and psychostimulant administration induces an enduring and progressive increase in the behavioural effects of subsequent drug injections. This long-lasting behavioural sensitization, including cross-sensitization between opiates and psychostimulants, has been postulated to play a role in the acquisition and maintenance of drug dependence (for reviews, see Wise and Bozarth 1987; Kalivas and Stewart 1991; Robinson and Berridge 1993; Stewart and Badiani 1993). It should, however, be emphasized that a causal relationship between behavioural sensitization and addiction behaviour has as yet not been demonstrated (see Altman et al. 1996). During the last decade, evidence has accumulated suggesting a crucial role for central dopaminergic and dopamine (DA)-sensitive neurons in behavioural sensitization. As drugs of abuse share the ability to activate forebrain DA neurons (Di Chiara and Imperato 1988), the expression of behavioural sensitization induced by psychostimulants and opiates appeared to be associated with an enhancement of drug- and depolarization-induced DA release in the nucleus accumbens as well as in the caudate-putamen of rat striatum in vivo and in vitro (Robinson et al. 1988; Kalivas and Stewart 1991; Patrick et al. 1991; Tjon et al. 1994; Nestby et al. 1995; Paulson and Robinson 1995; Heidbreder et al. 1996). In addition to an enhanced responsiveness of central DA neurons, enduring postsynaptic changes in DA-sensitive striatal neurons may underlie the long-term expression of behavioural sensitization. Thus, morphine-induced long- term behavioural sensitization appeared to be associated with an enhanced reactivity of DA-sensitive cholinergic

interneurons towards depolarization (Schoffelmeer et al. 1995a; Tjon et al. 1995) and glutamate receptor activation (Nestby et al. 1995). In addition, we recently showed that morphine-induced long-term behavioural sensitization to morphine and cross-sensitization to amphetamine is associated with supersensitivity of postsynaptic DA D_1 receptors and enhanced steady state levels of (D_1 receptor/cyclic AMP-dependent) prodynorphin gene expression in striatal gamma-aminobutyric acid neurons (Tjon et al. 1997). Moreover, neurophysiological studies showed that repeated psychostimulant administration may cause a long-lasting increase in the sensitivity of postsynaptic D_1 receptors mediating hyperpolarization of these striatal neurons (Wolf et al. 1994). An interesting feature of some of these drug-induced neuroadaptive changes in dopaminergic and DA-sensitive neurons is their delayed occurrence after cessation of repeated drug treatment (Kalivas and Duffy 1993; Tjon et al. 1994, 1997; Paulson and Robinson 1995; Heidbreder et al. 1996). Therefore, distinct neuroadaptations in the forebrain may underlie short- and long-term behavioural sensitization.

Assuming that long-term behavioural (cross)-sensitization is involved in the pathogenesis of addiction in general, it might be expected that the expression of the phenomenon is not only apparent long after repeated exposure to morphine or psychostimulants, but also after prior ethanol administration. However, although repeated injections of ethanol was shown to induce a sensitized locomotor response to ethanol in mice (Masur and Boerngen 1980; Masur et al. 1986; Roberts et al. 1995), this did not appear to occur in rats (Masur et al. 1986; Gingras and Cools 1996). The possibility that prior ethanol exposure may cause an enhanced locomotor effect of subsequent administration of psychostimulants or opiates has thus far not been studied. Furthermore, the occurrence of cross-sensitization between amphetamine, cocaine and opiates suggests the involvement of common neuroadaptive changes in the brain induced by these drugs (Robinson and Berridge 1993). However, whereas ethanol, like psychostimulants and morphine, is well known to acutely enhance striatal DA neurotransmission (Di Chiara and Imperato 1988; Weiss et al. 1993; Bassareo et al. 1996), the long-term effects of ethanol administration on the responsiveness of dopaminergic and DA-sensitive neurons have not been studied until now. Therefore, we investigated the locomotor effect induced by morphine and amphetamine and the reactivity of nucleus accumbens dopaminergic and cholinergic neurons towards depolarization, 3 weeks after repeated ethanol pretreatment.

Materials and methods

Animals and housing conditions

Male Wistar rats (Harlan, Zeist, The Netherlands), weighing 200–240 g at the beginning of drug treatment, were housed in Macrolon cages (two per cage), on a 12-h light/dark cycle (lights on at 7.00 a.m.). Standard food (Hope Farms, Woerden, The Netherlands) and tap water were available ad libitum. Upon arrival in the animal house, the animals were allowed to accustom to the housing facilities for 2 weeks. The animals were handled briefly for 3 days preceeding the beginning of drug treatment. All experiments were approved by the Animal Care Committee of the Free University of Amsterdam.

Drug treatment regimens

Animals were pretreated intraperitoneally (IP) with a dose of 1 g/kg ethanol, in a concentration of 10% (w/v) ethanol (10 ml/kg), once daily for 15 days, according to the protocol used by Bozarth (1990). Respective controls received IP injections of 10 ml/kg saline. Preliminary experiments revealed that an IP injection of 0.5–2 g/kg ethanol did not cause an appreciable locomotor effect in Wistar rats. In contrast, SC injection of 1–10 mg/kg morphine dose-dependently enhanced locomotor activity (see also Vanderschuren et al. 1997). Moreover, in a preliminary dose-response study, IP injections of 0.5–3 mg/kg amphetamine appeared to cause a dose-dependent increase in locomotion with a half-maximally effective dose of 1 mg/kg. Therefore, after 3 weeks withdrawal from ethanol or saline, two groups of rats were challenged with 1 g/kg ethanol ($n = 16$), 5 mg/kg morphine ($n = 16$) or 1 mg/kg amphetamine ($n = 16$), and the horizontal distance travelled was determined. The other rats ($n = 32$) were decapitated for the in vitro measurement of neurotransmitter release.

In a separate set of experiments, three additional groups of rats were repeatedly treated with morphine, cocaine or amphetamine. Respective control rats were treated with saline. As in our previous studies (Tjon et al. 1994; Vanderschuren et al. 1997), repeated morphine treatment consisted of one daily SC injection with morphine (10 mg/kg) for 14 days. Repeated cocaine consisted of one daily IP injection of cocaine (15 mg/kg) for 5 days, according to the treatment regimen of Kalivas and Duffy (1993). Sensitization to the locomotor effect of amphetamine was induced by one daily IP injection of amphetamine (2.5 mg/kg) for 5 consecutive days, as described previously (De Vries et al. 1996). Three weeks later, these rats were decapitated for in vitro measurement of neurotransmitter release.

Locomotor measurement

Horizontal motor activity was measured in Perspex cages ($40 \times 40 \times 40$ cm) using a video tracking system (EthoVision, Noldus Information Technology B.V., Wageningen, The Netherlands). All experiments were conducted between 9.00 a.m. and 15.30 p.m. White noise was produced by a noise generator. Animals were allowed to habituate to the test cages for 2 h, during which activity was monitored. After habituation, animals were injected with saline and activity was monitored for 1.5 or 2 h. Subsequently, animals were injected with ethanol (1 g/kg, 10 ml/kg, IP), morphine (5 mg/kg, 1 ml/kg, SC) or amphetamine (1 mg/kg, 1 ml/kg, IP). After the drug injection, horizontal activity was measured for 3 h (ethanol, morphine) or 1.5 h (amphetamine).

Determination of neurotransmitter release

Rats were decapitated 3 weeks after the last drug (ethanol, morphine, cocaine or amphetamine) injection. The caudate-putamen or nucleus accumbens were rapidly dissected from the brain. Tissue slices ($0.3 \times 0.3 \times 2$ mm) were prepared using a McIlwain tissue chopper. Slices (pooled tissue of four rats) were washed twice with Krebs-Ringer bicarbonate medium containing 121 mM NaCl, 1.87 mM KCl, 1.17 mM KH_2PO_4, 1.17 mM $MgSO_4$, 1.22 mM

$CaCl_2$, 25 mM $NaHCO_3$, 10 mM D-(+)-glucose and subsequently incubated for 15 min in this medium containing in a constant atmosphere of 95% O_2 −5% CO_2 at 37°C. After preincubation, the slices were rapidly washed with Krebs-Ringer and incubated for 15 min in 2.5 ml of this medium containing 5 µCi [^{3}H]DA and 2 µCi [^{14}C]choline in an atmosphere of 95% O_2 −5% CO_2 at 37 °C. The nucleus accumbens, in contrast to the caudate-putamen, has a dense noradrenergic innervation. Therefore, 3 µM desipramine was added to the medium during incubation of this brain region to prevent accumulation of [^{3}H]DA in noradrenergic nerve terminals. After labelling, the slices were rapidly washed and transferred to each of 24 chambers of a superfusion apparatus (approximately 4 mg tissue in 0.2 ml volume) and superfused (0.20 ml/min) with medium gassed with 95% O_2 −5% CO_2 at 37°C.

In each experiment, neurotransmitter release from brain slices of drug- and saline pretreated rats was studied simultaneously in 24 parallel superfusion chambers. The superfusate was collected as 10-min samples after 40 min of superfusion (t = 40 min). Ca^{2+}-dependent neurotransmitter release was induced during superfusion by exposing the slices to electrical biphasic block-pulses (1 Hz, 30 mA, 4 ms pulses) for 10 min at t = 50 min (electrical-field stimulation). The radioactivity remaining at the end of the experiment was extracted from the tissue with 0.1 N HCl. The radioactivity in superfusion fractions and tissue extracts was determined by liquid scintillation counting.

The efflux of radioactivity during each collection period was expressed as a percentage of the amount of radioactivity in the slices at the beginning of the respective collection period. The electrically evoked release of neurotransmitter was calculated by subtracting the spontaneous efflux of radioactivity from the total overflow of radioactivity during stimulation and the next 10 min. A linear decline from the 10-min interval before to that 20–30 min after the start of stimulation was assumed for calculation of the spontaneous efflux of radioactivity. The release evoked was expressed as percentage of the content of radioactivity of the slices at the start of the stimulation period.

Radiochemicals and drugs

Absolute (99.8%) ethanol was purchased from Baker B.V. (Deventer, The Netherlands). Morphine HCl, *d*-amphetamine HCl and cocaine HCl were obtained from O.P.G. (Utrecht, The Netherlands). [^{3}H]Dopamine (specific activity 47 Ci/mmol) and [^{14}C]choline (15 mCi/mmol) were obtained from the Radiochemical Centre (Amersham, United Kingdom). Desipramine (DMI) was purchased from Sigma (St Louis, MO., USA).

Statistics

Horizontal locomotor activity, expressed as distance travelled (in cm), was calculated in 10-min intervals and analyzed using two-factor repeated measures analyses of variance (ANOVA) for time and pretreatment. In the superfusion experiments, in vitro neurotransmitter release was calculated as percentage of the control values in the respective experiment (release upon saline pretreatment). Observations of different experiments were then pooled and analyzed using the two-tailed Student's *t*-test.

Results

Effect of repeated injections of ethanol on locomotor activity

In order to investigate the possible occurrence of long-term behavioural (cross-)sensitization upon prior repeated ethanol treatment, horizontal activity was determined in saline- and ethanol-pretreated rats following challenges with ethanol, morphine or amphetamine, 3 weeks after cessation of pretreatment. As shown in Fig. 1, the horizontal distance travelled following a saline challenge was not affected by prior ethanol treatment [$F(1, 14) = 0.37$, NS]. Preliminary experiments revealed that repeated ethanol exposure did not alter the slight locomotor effect of a second saline injection 90 or 120 min after the first injection (data not shown). The ethanol challenge given 120 min after the saline injection only slightly increased locomotor activity during the first minutes after its administration, and the horizontal distance travelled following the ethanol challenge did not differ between saline- and ethanol-pretreated rats [$F(1, 14) = 0.00$, NS].

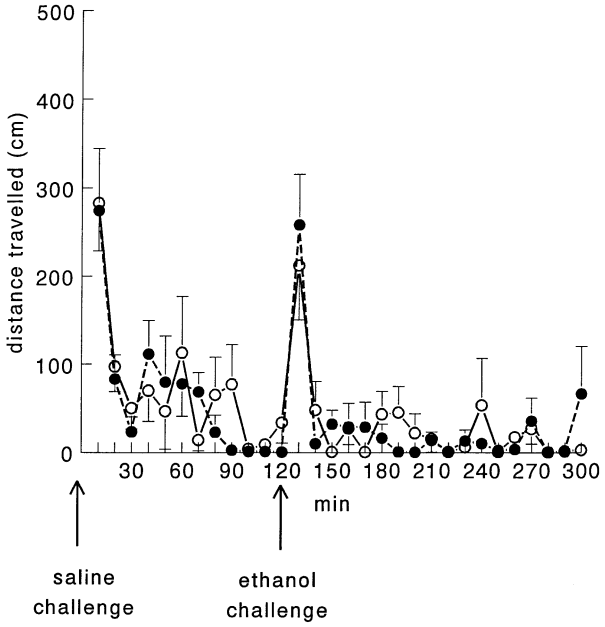

Fig. 1 Time-course of the locomotor response to an ethanol (1 g/kg) challenge in rats, 3 weeks following cessation of repeated ethanol ($n = 8$) (●) or saline ($n = 8$) (○) pretreatment. After habituation to the test cages, the animals were injected IP with saline, and 2 h later with 1 g/kg IP ethanol, and activity was monitored by videotracking for 3 h. Data are expressed as mean distance travelled (in cm) ± SEM per 10-min interval

As shown in Fig. 2, a challenge dose of 5 mg/kg morphine induced the well-known biphasic locomotor response in saline-pretreated rats, consisting of an initial sedative effect followed by a phase of hyperactivity (Vanderschuren et al. 1997, and references quoted therein). Morphine-induced locomotion was significantly enhanced in ethanol-pretreated rats compared to controls [$F(1, 14) = 7.20$, $P < 0.02$]. Locomotion following a saline challenge given 90 min prior to the morphine challenge was not different between the groups [$F(1, 14) = 0.34$, NS].

Amphetamine, at its half-maximally effective dose of 1 mg/kg, induced locomotor activation, the magnitude of which did not differ between saline- and ethanol

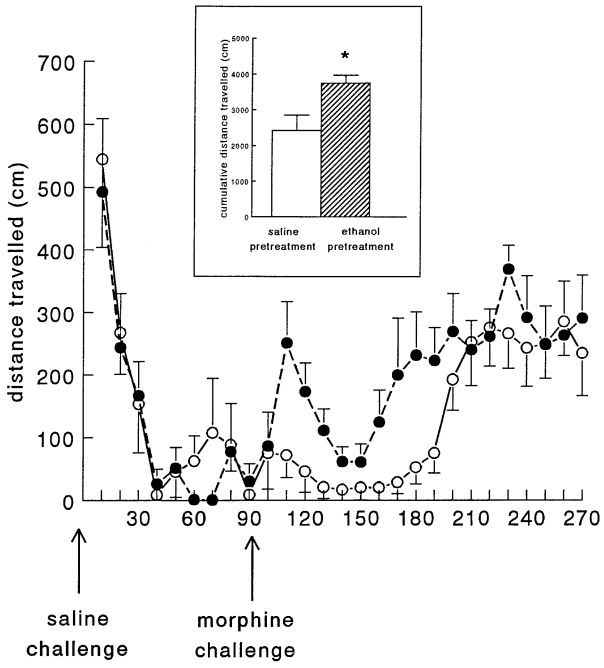

Fig. 2 Time-course of the locomotor response to a morphine (5 mg/kg) challenge in rats, 3 weeks following cessation of repeated ethanol ($n = 8$) (●) or saline ($n = 8$) (○) pretreatment. After habituation to the test cages, the animals were injected SC with saline, and 1.5 h later with 5 mg/kg SC morphine, and activity was monitored by videotracking for 3 h. Data are expressed as mean distance travelled (in cm) ± SEM per 10-min interval. The *inset* shows the cumulative distance travelled (in cm) ± SEM after the morphine challenge. $*P < 0.02$ (Student's *t*-test)

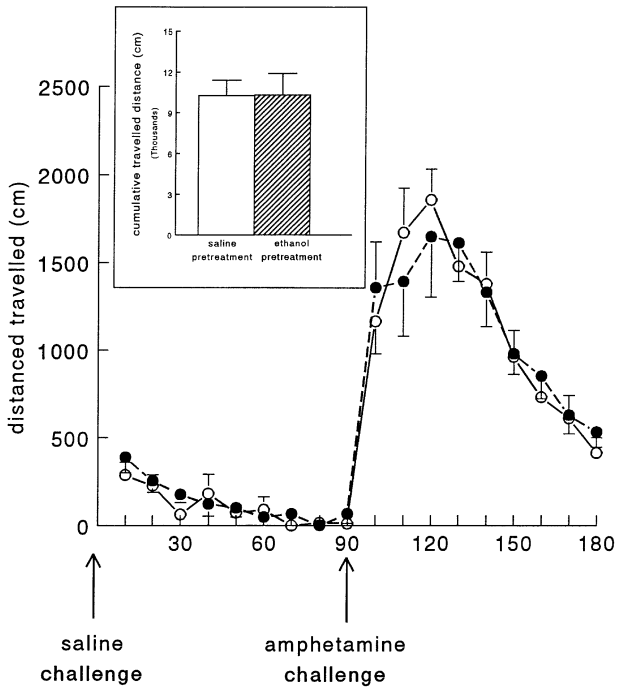

Fig. 3 Time-course of the locomotor response to an amphetamine (1 mg/kg) challenge in rats, 3 weeks following cessation of repeated ethanol ($n = 8$) (●) or saline ($n = 8$) (○) pretreatment. After habituation to the test cages, the animals were injected IP with saline, and 1.5 h later with 1 mg/kg IP morphine, and activity was monitored by videotracking for 1.5 h. Data are expressed as mean distance travelled (in cm) ± SEM per 10-min interval. The *inset* shows the cumulative distance travelled (in cm) ± SEM after the amphetamine challenge

pretreated rats, 3 weeks after the cessation of pretreatment [$F(1, 14) = 0.00$, NS] (Fig. 3). Locomotion following a saline challenge was not affected by prior ethanol treatment [$F(1, 14) = 1.10$, NS] as well.

Effect of repeated ethanol administration on electrically evoked [^{3}H]DA and [^{14}C]ACh release from nucleus accumbens slices

The long-term effect of repeated ethanol pretreatment on the electrically evoked [^{3}H]DA and [^{14}C]ACh release from superfused slices of rat nucleus accumbens was investigated 3 weeks after cessation of ethanol administration. The electrically stimulated [^{3}H]DA and [^{14}C]ACh release in excess of spontaneous [^{3}H] and [^{14}C] efflux amounted to, respectively, 0.62 ± 0.04% (SEM) and 3.32 ± 0.23% of total tissue radioactivity. Ethanol pretreatment significantly enhanced the electrically stimulated release of both neurotransmitters from slices of the nucleus accumbens. [^{3}H]DA release from slices of ethanol-pretreated rats was 33 ± 9% (SEM) higher than that from electrically stimulated slices of saline-pretreated rats ($t = 3.21$; $P < 0.005$), while the release of [^{14}C]ACh was increased by 55 ± 11% ($t = 4.82$; $P < 0.001$) (Fig. 4). Spontaneous [^{3}H] and [^{14}C] efflux from nucleus accumbens slices of saline-

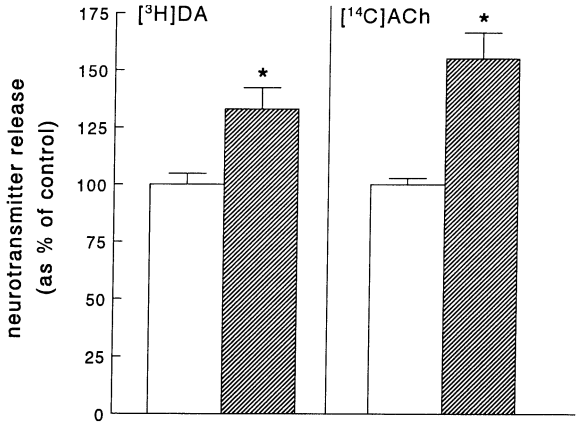

Fig. 4 Long-term effect of repeated ethanol pretreatment on the electrically evoked [^{3}H]DA and [^{14}C]ACh release from slices of rat nucleus accumbens. Three weeks after ethanol exposure, the slices were incubated with [^{3}H]DA and [^{14}C]choline and subsequently superfused. Neurotransmitter release was induced by means of electrical field-stimulation for 10 min. In each experiment, the release of both neurotransmitters from the slices of saline- and ethanol-pretreated rats was determined simultaneously. Results are expressed as percentage of control release from slices of saline-pretreated rats. Values represent means ± SEM of 24 observations obtained in four separate experiments. In each experiment tissue of four rats was pooled. $*P < 0.001$ versus control (Student's *t*-test). □ Saline, ▨ ethanol

Table 1 Common long-term effects of repeated ethanol, morphine, cocaine and amphetamine pretreament on the electrically evoked [^{3}H]DA and [^{14}C]ACh release from slices of rat nucleus accumbens. Three weeks after drug exposure, the slices were incubated with [^{3}H]DA and [^{14}C]choline, and subsequently superfused. In each experiment, the electrically evoked neurotransmitter release from slices of saline- and drug-pretreated rats was determined simultaneously. Results are expressed as percentage of control release from tissue of rats pretreated with saline. Values represent means ± SEM of 16–24 observations obtained in three or four separate experiments. In each experiment tissue of four rats was pooled

Pretreatment	[^{3}H]DA release (% of saline)	[^{14}C]ACh release (% of saline)
Ethanol	133 ± 9***	155 ± 11***
Morphine	181 ± 10***	149 ± 6***
Cocaine	113 ± 5*	125 ± 4**
Amphetamine	149 ± 12**	156 ± 5***

*$P < 0.05$ versus control; **$P < 0.01$; ***$P < 0.001$ (Student's t-test)

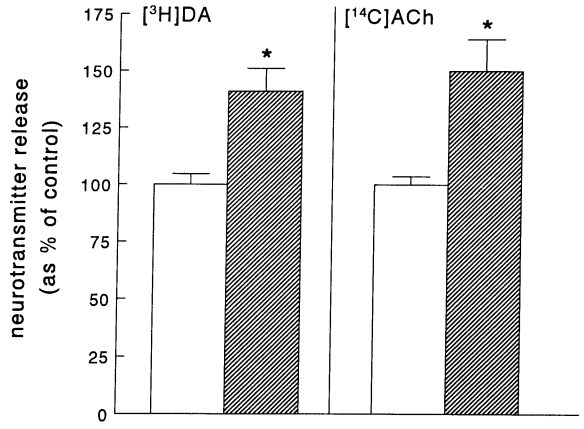

Fig. 5 Long-term effect of repeated ethanol pretreatment on the electrically evoked [^{3}H]DA and [^{14}C]ACh release from slices of rat caudate-putamen. Three weeks after ethanol exposure, the slices were incubated with [^{3}H]DA and [^{14}C]choline and subsequently superfused. Neurotransmitter release was induced by means of electrical field-stimulation for 10 min. In each experiment, the release of both neurotransmitters from the slices of saline- and ethanol-pretreated rats was determined simultaneously. Results are expressed as percentage of control release from slices of rats pretreated with saline. Values represent means ± SEM of 24 observations obtained in four separate experiments. In each experiment tissue of four rats was pooled. *$P < 0.001$ versus control (Student's t-test). □ Saline, ▨ ethanol

pretreated rats amounted to 2.5% of total radioactivity and was not affected by prior ethanol pretreatment.

Stimulus- and region-specificity of the long-term neuroadaptive effects

In order to investigate the stimulus specificity of the observed ethanol-induced increase of the electrically evoked increase in accumbal [^{3}H]DA and [^{14}C]ACh release, the effects of prior morphine, cocaine and amphetamine exposure on the release of these neurotransmitters was also examined, 3 weeks after cessation of drug treatment. Prior repeated morphine, as well as cocaine or amphetamine treatment, significantly enhanced the electrically evoked release of [^{3}H]DA and [^{14}C]ACh (Table 1). The electrically evoked release of neurotransmitters from accumbal slices of saline-pretreated rats did not differ from that observed in the ethanol experiments. Spontaneous efflux of [^{3}H] and [^{14}C] release in slices of the nucleus accumbens was not affected by prior morphine, amphetamine or cocaine treament.

The ethanol-induced long-term neuroadaptations did not appear to be specific for the nucleus accumbens, since similar ethanol-induced effects were also observed in slices of the caudate-putamen. Three weeks after saline pretreatment, the electrically stimulated [^{3}H]DA release from slices of caudate-putamen amounted to 1.10 ± 0.07% (SEM) and the [^{14}C]ACh release amounted to 4.75 ± 0.31% of total tissue radioactivity in excess of spontaneous efflux of radioactivity. Repeated ethanol pretreatment significantly increased electrically evoked [^{3}H]DA and [^{14}C]ACh release from slices of caudate-putamen by 41 ± 10% (SEM) ($t = 3.6$; $P < 0.001$) and 50 ± 14% ($t = 3.56$; $P < 0.001$), respectively (Fig. 5).

Discussion

The present study shows that repeated administration of ethanol induces an increase in morphine-induced locomotor activity, 3 weeks after the last ethanol injection. Thus, long-term behavioural (cross-)sensitization may not only occur upon prior exposure to psychostimulants and opiates (Robinson and Berridge 1993; Stewart and Badiani 1993), but also after repeated ethanol treatment. In agreement with previous studies (Frye and Breese 1981; Masur et al. 1986), ethanol exposure did not cause a major locomotor effect in Wistar rats. Apparently, the occurrence of an acute locomotor effect does not seem to be required for a drug to induce behavioural sensitization. In this respect, it has also been shown that administration of amphetamine into the ventral tegmental area does not induce a locomotor effect, whereas it causes locomotor sensitization to subsequent peripheral injections of morphine, amphetamine or cocaine (Kalivas and Weber 1988; Vezina and Stewart 1990; Hooks et al. 1992; Vezina 1993). The long-term expression of ethanol-induced behavioural (cross-)sensitization to morphine observed here suggests that ethanol administration causes long-lasting neuroadaptations in the brain that may show similarities to those previously observed for other addictive drugs.

During the last decade, it has become clear that dopaminergic neurons and DA-sensitive neurons play a crucial role in behavioural sensitization (see

Introduction), the initiation and expression of which may occur in distinct parts of the brain. Activation of the cell bodies of dopaminergic neurons and DA D_1 receptors in the ventral tegmental area has been suggested to be crucial for the induction of behavioural sensitization (Vezina et al. 1987; Perugini and Vezina 1994; Cador et al. 1995; Pierce et al. 1996; Vezina 1996). In contrast, the expression of behavioural sensitization is associated with altered dopaminergic neurotransmission processes in the projection areas of mesocorticolimbic dopaminergic neurons (Kalivas and Stewart 1991; Kalivas and Duffy 1993; Cador et al. 1995). With regard to the expression of drug-induced locomotor sensitization, there is strong evidence that dopaminergic neurotransmission in the rat nucleus accumbens plays a pivotal role (for review, see Kalivas et al. 1993). Since our behavioural experiments showed long-term sensitization of morphine-induced locomotion after repeated ethanol treatment, our neurochemical studies focused on ethanol-induced long-term effects in this part of the striatum, comparing protracted changes in neuronal responsiveness with those observed long after repeated morphine, cocaine and amphetamine pretreatment.

With regard to the reactivity of rat striatal neurons, we recently reported that morphine-induced behavioural sensitization is associated with an adaptive increase in the reactivity of dopaminergic nerve terminals and cholinergic interneurons in caudate-putamen slices towards depolarization (Tjon et al. 1994, 1995; Schoffelmeer et al. 1995b). The present study shows that prior repeated morphine administration for 14 days, inducing long-lasting sensitization towards the locomotor effect of morphine (Vanderschuren et al. 1997), also causes such an increase in the electrically evoked release of [^{3}H]DA and [^{14}C]ACh from superfused slices of the nucleus accumbens. Since repeated ethanol treatment resulted in a sensitized locomotor effect of morphine 3 weeks later, it might be expected to induce similar neuroadaptations in the nucleus accumbens. Indeed, the present study shows that such neuroadaptative changes in the nucleus accumbens are also apparent after repeated administration of ethanol. The observation of similar changes in the reactivity of nucleus accumbens dopaminergic and cholinergic neurons, subsequent to repeated cocaine and amphetamine exposure, indicates that these neuroadaptations are shared by pharmacologically distinct drugs of abuse. Therefore, our data indicate that an enhanced responsiveness of dopaminergic nerve terminals and DA-sensitive cholinergic neurons towards depolarization represents a common long-term neuroadaptive effect induced by pharmacologically distinct addictive drugs. In addition, our study shows that the ethanol-induced neuroadaptations are not restricted to the nucleus accumbens, but are also apparent in the caudate-putamen as previously observed after repeated morphine treatment (Tjon et al. 1994, 1995; Schoffelmeer et al. 1995) and repeated cocaine or amphetamine treatment (data not shown). This is of interest, since the caudate-putamen also receives dopaminergic innervation from the ventral mesencephalon and is considered to play a modulatory role in behavioural sensitization (for review, see Kalivas et al. 1993).

It is possible that the occurrence of an enhanced reactivity of DA and DA-sensitive striatal neurons long after repeated exposure to opiates, psychostimulants or ethanol is a direct consequence of repeated activation of DA neurons. On the other hand, it is also conceivable that these enduring neuroadaptations are primarily caused by distinct common effects of addictive drugs, such as their ability to activate the hypothalamus-pituitary-adrenal axis (Pfeiffer and Herz 1984; Rivier and Vale 1987; Swerdlow et al. 1993; Roberts et al. 1995). In this respect, we recently showed that activation of corticosteroid receptors by corticosterone may exert a facilitatory action on DA neurotransmission in rat striatum (Ronken et al. 1994; Schoffelmeer et al. 1995, 1996) and the expression of long-term behavioural sensitization (De Vries et al. 1996).

Considering the drug-induced enhanced reactivity of cholinergic interneurons, it is of interest that an enhanced cholinergic neurotransmission upon prior morphine exposure was recently shown to cause an increase in DA release in the caudate-putamen through activation of presynaptic muscarinic receptors in dopaminergic nerve terminals (Schoffelmeer et al. 1995). In addition, enhanced activation of presynaptic nicotine receptors by released ACh may cause partial depolarization of dopaminergic neurons, enhancing their excitability (Lichtensteiger et al. 1982; Lacey et al. 1990). In view of the crucial role of cholinergic interneurons in the regulation of afferent, intrinsic and efferent neurons of the caudate-putamen and nucleus accumbens (for review, see Di Chiara et al. 1994), an enhanced reactivity of striatal cholinergic interneurons induced by repeated administration of addictive drugs, may be of particular relevance for the long-term expression of behavioural (cross)-sensitization.

However, in contrast to the occurrence of locomotor sensitization to morphine, we found that the amphetamine-induced locomotor response did not differ between saline- and ethanol-pretreated rats, 3 weeks after cessation of treatment. We previously showed that the locomotor effect of amphetamine (1 mg/kg) was strongly enhanced in rats, 3 weeks after repeated exposure to morphine (Vanderschuren et al. 1997). Apparently, the neuroadaptive changes induced by prior ethanol treatment are not sufficient to cause a sensitized locomotor response to amphetamine. This might be related to the different acute cellular mechanisms of action of morphine and amphetamine. Morphine causes μ-opioid receptor mediated enhancement of action potential-induced exocytotic DA release, probably through inhibition of γ-aminobutyric acid neurons (Johnson and North 1992; Klitenick

et al. 1992). In contrast, amphetamine displaces vesicular DA in dopaminergic nerve terminals, independent of neuronal depolarization (for review, see Kuczenski and Segal 1994). This calcium-independent DA releasing action of amphetamine has been shown to be enhanced in amphetamine-pretreated rats (Castaneda et al. 1988). Our locomotor studies with ethanol-pretreated rats suggest that the expression of a sensitized response to amphetamine may at least partially be due to molecular neuroadaptations different from those underlying the expression of locomotor sensitization to morphine.

In conclusion, our data indicate that repeated ethanol administration may cause long-term (cross-) sensitization to the locomotor effect of morphine in rats. This locomotor sensitization appeared to be associated with an increase in the reactivity of dopaminergic nerve terminals and DA-sensitive cholinergic neurons in rat nucleus accumbens and caudate-putamen. In view of the fact that similar long-term changes were observed in the nucleus accumbens of morphine-, amphetamine- and cocaine-pretreated rats, these common neuroadaptations may play a role in the long-term expression of behavioural sensitization in general, independent of the type of the addictive drug. However, the possible causal relationship between the long-term neuroadaptations shown here and the expression of behavioural sensitization requires further study. In this respect, the observation that the locomotor effect of an amphetamine challenge was not enhanced in ethanol-pretreated rats, indicates that ethanol-induced long-term neuroadaptations may not always be sufficient to cause behavioural (cross-)sensitization.

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