Opiate-Induced Dopamine Release Is Modulated by Severity of Alcohol Dependence: An [^{18}F]Fallypride Positron Emission Tomography Study

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Background: Preclinical data implicate the reinforcing effects of alcohol to be mediated by interaction between the opioid and dopamine systems of the brain. Specifically, alcohol-induced release of β-endorphins stimulates μ-opioid receptors (MORs), which is believed to cause dopamine release in the brain reward system. Individual differences in opioid or dopamine neurotransmission have been suggested to be responsible for enhanced liability to abuse alcohol. In the present study, a single dose of the MOR agonist remifentanil was administered in detoxified alcohol-dependent patients and healthy control subjects to mimic the β-endorphin-releasing properties of ethanol and to assess the effects of direct MOR stimulation on dopamine release in the mesolimbic reward system.

Methods: Availability of $D_{2/3}$ receptors was assessed before and after single-dose administration of the MOR agonist remifentanil in 11 detoxified alcohol-dependent patients and 11 healthy control subjects with positron emission tomography with the radiotracer [^{18}F]fallypride. Severity of dependence as assessed with the Alcohol Use Disorders Identification Test was compared with remifentanil-induced percentage change in [^{18}F]fallypride binding ($\Delta\%BP_{ND}$).

Results: The [^{18}F]fallypride binding potentials (BP_{ND}s) were significantly reduced in the ventral striatum, dorsal putamen, and amygdala after remifentanil application in both patients and control subjects. In the patient group, ventral striatum $\Delta\%BP_{ND}$ was correlated with the Alcohol Use Disorders Identification Test score.

Conclusions: The data provide evidence for a MOR-mediated interaction between the opioid and the dopamine system, supporting the assumption that one way by which alcohol unfolds its rewarding effects is via a MOR-(γ-aminobutyric acid)-dopamine pathway. No difference in dopamine release was found between patients and control subjects, but evidence for a patient-specific association between sensitivity to MOR stimulation and severity of alcohol dependence was found.

Key Words: Alcohol dependence, dopamine receptor availability, μ-opioid receptors, opioid-dopamine interaction, positron emission tomography, reward

Like other drugs of abuse, alcohol is assumed to exert its reinforcing effects via activation of the mesolimbic dopamine system (1). Preclinical data provide evidence that this activation involves increased firing of dopamine neurons in the ventral tegmental area (VTA) of the midbrain (2) and a subsequent increase of dopamine released into the striatum, specifically the nucleus accumbens (NAc) (3). In humans, evidence for alcohol-induced dopamine release in the striatum was provided by positron emission tomography (PET) studies, showing increases in tracer displacement from dopamine $D_{2/3}$ receptors after alcohol consumption in nonalcohol-dependent social drinkers (4–6), although some studies failed to find significant effects of alcohol on tracer binding (7,8), possibly as a consequence of small sample sizes. The mechanism by which alcohol triggers dopamine release is not yet fully understood. Ethanol does not act on dopamine neurons directly but is presumed to exert its effect via interaction with different neurotransmitter/neuropeptide systems (see Vengeliene *et al.* [9] for a review). One route of action involves the opioid system. Animal data have provided evidence that ethanol-induced β-endorphin release increases dopamine release in the NAc by activating μ-opioid receptors (MORs) in the VTA (10). The activation of the dopamine system by MORs seems to be mediated via inhibition of γ-aminobutyric acid (GABA)ergic neurons that normally hold the dopamine system under inhibitory control (11). It is not yet clear whether a similar mechanism is active in the human brain. One PET study tried to assess the effect of MOR stimulation on striatal dopamine release in a relatively small group of healthy participants (n = 8), reporting slight increases in $D_{2/3}$ receptor binding of [^{11}C]raclopride rather than the expected decrease after stimulation with the MOR agonist alfentanil (12). No changes in [^{11}C]raclopride binding were found in heroin addicts taking methadone maintenance treatment after MOR stimulation with diamorphine and hydromorphone (13).

However, evidence has accumulated in animals and humans, that liability to abuse alcohol is associated with changes in the opioid system. Specifically, greater sensitivity of pituitary β-endorphin to ethanol was found in alcohol-preferring rats (14). Although previous human studies failed to detect differences in plasma endorphin levels in alcohol-dependent individuals (15), enhanced β-endorphin release to ethanol was found in subjects with high risk

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for alcoholism (i.e., subjects from families with a history of alcoholism) compared with low-risk subjects (16). Also, increased MOR density/affinity was found in alcohol-dependent patients and could be linked to experiences of craving and risk to relapse after alcohol consumption was stopped (17).

With regard to dopamine, prior research has provided evidence that dopamine activity is blunted in alcohol-dependent patients. Reports of lowered dopamine D_2 receptor availability in the striatum (18,19) suggest reduced extracellular dopamine levels in alcohol-addicted patients, although not all studies replicated this finding (20). Studies addressing presynaptic dopamine function in detoxified alcohol-dependent patients and healthy control subjects via amphetamine/methylphenidate challenge reported decreased presynaptic dopamine release in the patient group (21,22). However, there is evidence that attenuated basal dopamine function, suggested not only in alcohol-dependent patients but also in individuals addicted to other drugs of abuse (23,24), might be contrasted by pathologically elevated dopamine release in response to the drug itself (25). This assumption is supported by preclinical data indicating elevated dopamine responses to ethanol administration in animals with chronic alcohol consumption as well as in alcohol-preferring rat lines compared with control animals, despite equal (26) or even lowered (27) baseline levels of extracellular dopamine.

Thus, it might be hypothesized that in alcohol-dependent patients greater sensitivity to MOR stimulation is also associated with increased dopamine release in the NAc, irrespective of dopamine function at baseline. In vivo microdialysis has revealed that administration of selective MOR agonists increases dopamine release in the NAc of the rat (28). However, differential effects with regard to alcohol consumption have not yet been assessed, nor has the effect been demonstrated in the human brain.

We used the MOR agonist remifentanil to test the effect of MOR stimulation on dopamine release in a group of recently detoxified alcohol-dependent patients and healthy comparison subjects. Positron emission tomography and the $D_{2/3}$ receptor selective tracer [^{18}F]fallypride were used to assess MOR agonist-induced dopamine release compared with baseline.

Methods and Materials

Subjects

The local ethics committee, the Federal Institute for Drugs and Medical Devices, and the Federal Office for Radiation Protection approved this study.

Eleven recently detoxified male alcohol-dependent patients (47.9 ± 7 years old, range 36–57 years) and 11 healthy male control subjects (45.4 ± 7 years old, range 36–57 years) were included in the study after providing written informed consent. None of the participants had been taking any psychotropic medication for at least 2 weeks. All patients fulfilled the diagnosis of alcohol dependence according to the ICD-10 and DSM-IV criteria and had no other psychiatric Axis I disorder, no history of drug dependence, and no current drug abuse (urine drug testing and Structured Clinical Interview for DSM-IV), except for nicotine abuse and caffeine consumption. Severity of alcohol dependence was assessed with the Alcohol Use Disorders Identification Test (AUDIT) published by the World Health Organization (2001). All patients participated in the detoxification program of the Department of Psychiatry, Psychotherapy and Psychosomatics at the Rheinisch-Westfälische Technische Hochschule Aachen University Hospital in Germany. Time since last drink at the time of scanning ranged between 8 and 48 days (median = 32 days), with the exception of one patient who had been abstinent for 126 days at the time of scanning (hence, medium duration of abstinence for the whole group = 36 days). Alcohol abstinence at the time of the scan was confirmed via urine test, analyzing ethyl glucuronide, which mirrors potential alcohol intake within the last 5 days.

All subjects were scanned twice with PET with the dopamine receptor radiotracer [^{18}F]fallyride, once at baseline and again 2 weeks later (mean = 2.4 ± 1.9) after remifentanil administration. Remifentanil was injected 5 min before [^{18}F]fallyride administration with the dose adjusted to participant weight (.3 μg/kg bodyweight). Craving for an alcoholic drink was assessed immediately before and after each scan session with the Alcohol Urge Questionnaire.

PET Scanning

The PET scans were acquired with a Siemens ECAT EXACT 922/47 whole-body PET scanner (CTI/Siemens, Knoxville, Tennessee) in three-dimensional mode (field-of-view: 16.2 cm; 47 planes; full width at half maximal axial: 4.6 mm, in-plane: 6.0 mm). The radiosynthesis of [^{18}F]fallypride was a high-yield modification of the method for the synthesis of [^{18}F]desmethoxyfallypride, as described in detail previously (29, 30). First, a 15-min transmission scan with a ^{68}Ge source was carried out for subsequent attenuation correction. Afterwards, a mean of 221 MBq (SD = 26) of [^{18}F]fallypride was injected intravenously as a bolus into a cubital vein over approximately 30 sec. The specific activity at the time of injection was 746 GBq/μmol (SD = 662), corresponding to an injected mass of .3 μg (SD = .7). The PET data were acquired over a duration of 180 min. For registration purposes, a volumetric magnetic resonance imaging (MRI) scan of the head of each participant was also performed.

The PET data analysis was performed with PMOD software (version 3.1, PMOD Technologies, Zurich, Switzerland). To correct for PET frame misalignment caused by head movement, the dynamic PET frames were first realigned to a reference summed image of a period with little movement (between 40 and 60 min) with the within modality Automatic Image Registration algorithm (31) as implemented in PMOD (PMOD Technologies). The motion-corrected dynamic PET dataset of each individual was coregistered (rigid body transformation) to the MRI of the same subject with a mutual information algorithm. The MRI was spatially normalized to the T1-weighted MRI template of the Montreal Neurological Institute with nonlinear warping. The obtained transformation parameters were applied to the corresponding PET dataset. Parametric images of nondisplaceable binding potentials (BP_{ND}) were generated from the dynamic PET scans, with a basis function implementation of the simplified reference region compartmental model (32,33). The BP_{ND} refers to the ratio at equilibrium of specifically bound radioligand to that of nondisplaceable radioligand in tissue. The cerebellum was chosen as reference region. To assess differential effects of group and/or treatment on the mesolimbic reward system, a region-of-interest (ROI) analysis was performed. The ROIs were automatically defined in Montreal Neurological Institute space with a predefined template image based on the AAL template and applied to the coregistered PET image of each individual. The striatum was divided into ventral striatum, dorsal caudate, and dorsal putamen, following the method described by Mawlawi *et al.* (34).

The change in $D_{2/3}$ receptor availability as a result of change in endogenous dopamine concentration was calculated as the percentage change in binding potential with respect to nondisplaceable tracer concentration in the brain ($\%\Delta BP_{ND}$), with the following equation:

$$\%\Delta BP_{ND} = 100 * [(BP_{ND\ MOR\text{-}agonist} / BP_{ND\ baseline}) - 1].$$

Statistical Analysis

Group demographic comparisons were performed with unpaired *t* tests. Separate repeated-measures analyses of variance (ANOVAs) were performed on specific activities and injected masses with factors "group" (patients vs. control subjects) and "treatment" (scan 1 vs. scan 2). In the statistical analysis on [^{18}F]fallypride BP_{ND}s, only regions were included with a mean $BP_{ND} > 1.0$ to avoid errors due to a low signal-to-noise ratio. Data from five ROIs were included, following this approach, in the statistical analysis: ventral striatum, dorsal caudate, dorsal putamen, amygdala, and thalamus. To assess group differences in BP_{ND} at baseline, a multivariate ANOVA with ROI (ventral striatum, dorsal caudate, dorsal putamen, amygdala, thalamus) as within-subject factor and diagnostic group (patients, control subjects) as between-subject factor was performed on the data collected in the first scanning session (SPSS Statistics, Chicago, Illinois). Effects of MOR agonist administration (remifentanil) on [^{18}F]fallypride-BP_{ND} in the two groups were analyzed in a multivariate repeated-measures ANOVA with treatment (baseline, MOR agonist) and ROI (ventral striatum, dorsal caudate, dorsal putamen, amygdala, thalamus) as within-subject factor and diagnostic group (patients, control subjects) as between-subject factors. In both analyses, the Huynh-Feldt-ε-correction was used in the event of violations of sphericity assumptions. Post hoc comparisons were performed with paired *t* tests with Bonferroni correction for multiple comparisons. In all analyses, a two-tailed probability value of $p < .05$ was chosen as statistically significant. Effect size of significant results is reported with partial η-squared (η_p^2). To test for a relationship between drinking behavior and sensitivity to opiate stimulation, Pearson product-moment correlations were calculated between AUDIT score and $\%\Delta BP_{ND}$ in the defined ROIs, separately in patients and control subjects. All tests were additionally performed with age as a covariate (i.e., calculating partial correlations), to ensure that associations between AUDIT score and $\%\Delta BP_{ND}$ were not confounded with age effects.

Results

Demographic Data and Clinical Measures

Mean age was not different between patients and control subjects [47.9 ± 7 vs. 45.4 ± 7; $t(20) = .9$, $p > .05$]. Measures of dependence severity were significantly higher in alcohol-dependent patients than control subjects (Table 1). In the patient group, no significant correlation was found between AUDIT score and days of abstinence ($p > .05$).

Specific activities and injected mass did not differ significantly between patients and control subjects; nor were they significantly different at scan 1 and 2 (all $p > .05$) (Table 1).

Table 1. Demographic Data and Clinical Measures

Variable	Alcohol-Dependent Patients	Healthy Control Participants
Age	47.9 ± 7	45.4 ± 7
AUDIT Score[a]	24.8 ± 9	4.3 ± 3
Number of Drinking Days/Week[a]	3.6 ± 1	2.4 ± 1
Number of Alcoholic Drinks/Day[a]	8.2 ± 2	3.6 ± 3
Number of Smokers/Group	4/11	4/11
[^{18}F]Fallypride Injected Activity (MBq)		
Scan1	223.2 ± 34.1	227.5 ± 25.1
Scan 2	213.5 ± 23.0	218.4 ± 24.8
[^{18}F]Fallypride Specific Activity (GBq/μmol)		
Scan1	849.9 ± 673.1	707.7 ± 840.7
Scan 2	742.7 ± 840.7	692.9 ± 563.6
[^{18}F]Fallypride Injected Mass (μg)		
Scan1	.19 ± .2	.64 ± 1.3
Scan 2	.24 ± .3	.22 ± .2

Mean ± SD.
AUDIT, Alcohol Use Disorders Identification Test.
[a]Significant ($p < .05$).

Craving Assessment

Reported craving was not significantly different between patients and comparison subjects at any time point (all comparisons $p > .05$).

PET Data Analysis

$D_{2/3}$ Receptor Availability at Baseline. The multivariate ANOVA on baseline data (collected during the first scan) revealed no significant effect of group or group × region interaction ($p > .05$). No significant correlation was found between baseline BP_{ND} and AUDIT score of patients or between baseline BP_{ND} and days of abstinence of patients (both $p > .05$).

Effects of MOR Agonist Administration on $D_{2/3}$ Receptor Availability. The repeated-measures ANOVA revealed a main effect of treatment with remifentanil [factor treatment: $F(1,20) = 13.55$, $p = .001$, $\eta_p^2 = .41$], which interacted with region [treatment × region interaction: $F(4,80) = 9.61$, $p = .001$, $\eta_p^2 = .33$], reflecting significantly reduced dopamine $D_{2/3}$ receptor availability after administration of the MOR agonist remifentanil compared with baseline in the ventral striatum (−9.5%), the dorsal putamen (−8.3%), and the amygdala (−12.5%) (Figure 1, Table 2).

There was no significant group × treatment [$F(1,20) = 1.3$, $p = .27$] or group × treatment × region interaction [$F(4,80) = .97$, $p = .37$].

Relationship Between MOR Agonist-induced Percentage Change in BP_{ND} ($\%\Delta BP_{ND}$) and AUDIT Score

A significant correlation between $\%\Delta BP_{ND}$ and AUDIT score was only seen in the ventral striatum and was restricted to the patient group ($r = -.62$, $p = .02$), reflecting an increase of $\%\Delta BP_{ND}$ with increasing severity of alcohol dependence (Figure 2). The correlation remained statistically significant after controlling for age ($r = -.56$, $p = .046$). No significant correlation was found between $\Delta\%BP_{ND}$ and AUDIT score or $\Delta\%BP_{ND}$ and days of abstinence, in any region (all $p > .05$).

Discussion

The primary goal of this study was to examine the effects of MOR stimulation on dopamine release in detoxified alcohol-dependent patients compared with healthy control subjects. The MOR agonist remifentanil was used to mimic the effect of β-endorphin release after ethanol consumption. Activation of MORs is presumed, on the basis of rodent data, to inhibit GABAergic neurons in the VTA (11). Assuming that VTA dopamine neurons are under the tonic control of VTA GABA-neurons (35), blockade of GABAergic innervation is believed to increase the activity of VTA dopamine neurons, hence, causing enhancement of dopamine release in VTA projection areas (10).

In a group of 11 patients and 11 control subjects, we found a significant BP_{ND} reduction after stimulation with remifentanil in subregions of the striatum and in the amygdala. The reduction in [^{18}F]fallypride BP_{ND} is consistent with an increase in dopamine levels in these regions.

This is the first study demonstrating MOR stimulation–induced dopamine release in the human mesolimbic reward system. The

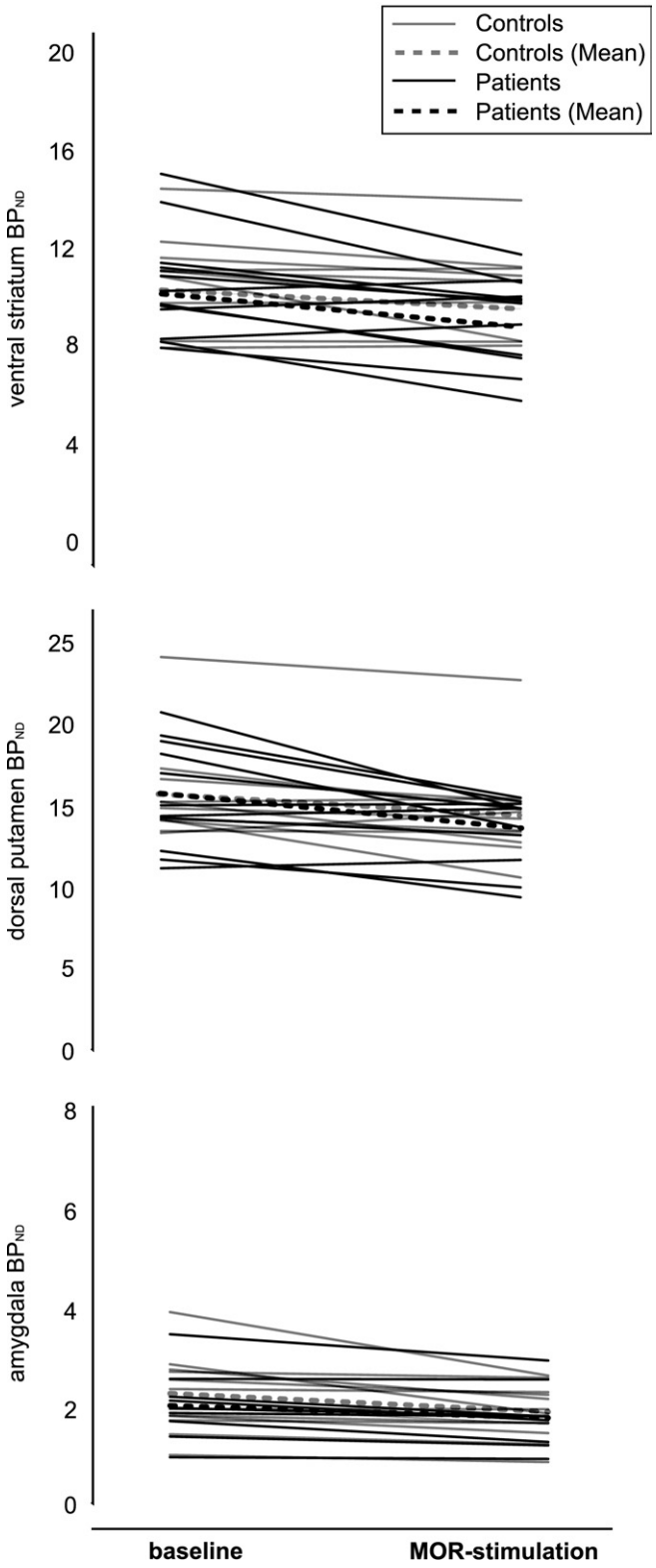

Figure 1. Individual changes in ventral striatum nondisplaceable binding potentials (BP_{ND}) (top), dorsal putamen (middle), and amygdala (bottom) at baseline and after stimulation with remifentanil in patients (black lines) and control subjects (grey lines). Group mean changes are depicted as dotted lines. MOR, μ-opioid receptor.

result is in line with preclinical findings in rodents (11,28) and provides further evidence for the existence of an (most likely GABA-mediated) interaction cascade between MORs and dopaminergic projections in limbic brain areas (10,36). It was hypothesized that dopamine release induced via MOR stimulation would differ between patients and control subjects. The group comparison did not yield a significant difference between patients and control subjects. However, in the patient group, the relative decrease in $D_{2/3}$ receptor availability was associated with drinking severity (i.e., $\Delta\%BP_{ND}$ was larger in patients reporting past heavy drinking). This finding indicates that sensitivity of the MOR-(GABA)dopamine pathway to MOR stimulation is not equally pronounced among alcohol-dependent individuals. Rather, there seems to be an association between MOR-mediated dopamine response and severity of alcohol abuse. The finding is mirrored by reports that MOR blockade with the MOR antagonist naltrexone, successfully used as treatment to reduce craving and relapse rates in alcohol and opiate addicts, is unequally effective and differs in severity of side effects in different individuals (37,38). It has been suggested that these differences might be caused by individual variances in MOR density/affinity (38). A recently detected association between a polymorphism of the MOR gene (*OPRM1*) and alcohol misuse (37,39) seems to support this notion. Effectiveness of naltrexone to reduce craving and relapse rates by blocking MORs was more pronounced in alcohol-dependent individuals carrying a variant of the *OPRM1* gene (A118G single nucleotide polymorphism) that has been associated with greater alcohol consumption and preference (40).

In the present study we did not assess MOR availability directly. However, Heinz *et al.* (17) reported higher MOR availability in detoxified alcohol-dependent patients than in comparison subjects, which also correlated with experienced craving. It can be speculated, assuming a link between craving and drinking severity (41), that the association between remifentanil-induced dopamine release and dependence severity in our sample can be attributed to a greater number of MORs (i.e., greater sensitivity to MOR stimulation) in heavy drinkers. Of course, the finding that MOR stimulation with a MOR-specific agonist stimulates pronounced dopamine release in alcohol-dependent individuals does not necessarily mean that alcohol would have the same effect. However, a recent PET study with social drinkers revealed alcohol-induced striatal dopamine release to be particularly pronounced in individuals carrying the A118G single nucleotide polymorphism of the *OPRM1* gene (42), providing yet another piece of evidence for a link between heightened MOR sensitivity and increased dopamine release in response to alcohol consumption.

Our finding of reduced BP_{ND} in striatal subregions somewhat differs from the finding by Hagelberg *et al.* (12). The authors reported a small increase in tracer binding after stimulation with alfentanil in a group of nonalcohol-dependent participants. Their regions of interest encompassed the caudate nucleus and the putamen, without further regional subdivision. In our study, no significant effect was observed in the dorsal caudate, but a decrease was observed in the ventral striatum and the dorsal putamen. In the light of massive differences in direction and extent of dopaminergic projections to striatal subdivisions (43), methodological differences in ROI partition might well cause divergent findings (34). Also, it cannot be ruled out that differences in the pharmacokinetics of the two opioids (44) as well as differences in the administration protocol and/or PET tracer characteristics led to different effects. Finally, it needs to be noted that, like Hagelberg *et al.*, we observed heterogeneous effects among our participants, and a decrease of BP_{ND} was not observed in all (Figure 1). The reasons for this interindi-

Table 2. Dopamine $D_{2/3}$ Receptor Availability at Baseline and After MOR Stimulation with Remifentanil

		Treatment Condition			
	Group[a]	Baseline BP_{ND} Mean ± SE	MOR-Stimulation BP_{ND} Mean ± SE	p^b	$\Delta\%BP_{ND}$ Mean ± SE
Ventral Striatum	Whole Group	10.62 ± .43	9.56 ± .40	<.001[c]	−9.51 ± 2.35
	Patients	10.54 ± .69	9.19 ± .55		−12.03 ± 3.85
	Control Subjects	10.69 ± .55	9.93 ± .57		−6.99 ± 2.65
Dorsal Caudate	Whole Group	14.03 ± .72	13.17 ± .61	.05	−4.53 ± 3.12
	Patients	13.63 ± 1.13	12.32 ± .82		−6.83 ± 5.9
	Control Subjects	14.43 ± .92	14.03 ± .87		−2.23 ± 3.17
Dorsal Putamen	Whole Group	15.93 ± .71	14.22 ± .62	<.001[c]	−8.26 ± 2.32
	Patients	15.96 ± .99	14.08 ± .70		−10.36 ± 3.76
	Control Subjects	15.90 ± 1.07	14.99 ± 1.07		−6.16 ± 2.75
Amygdala	Whole Group	2.17 ± .15	1.86 ± .12	<.001[c]	−12.46 ± 2.17
	Patients	2.30 ± .26	1.92 ± .19		−12.87 ± 3.21
	Control Subjects	2.05 ± .17	1.80 ± .15		−12.06 ± 3.07
Thalamus	Whole Group	2.09 ± .20	1.89 ± .18	.05	−5.55 ± 5.77
	Patients	1.89 ± .28	1.70 ± .26		−10.07 ± 5.75
	Control Subjects	2.29 ± .29	2.09 ± .25		−1.03 ± 10.13

MOR, μ-opioid receptor; BP_{ND}, nondisplaceable binding potentials; $\%\Delta BP_{ND}$, percentage change in nondisplaceable binding potential.

[a]No significant effect of group or group × region.

[b]Result of post hoc pairwise comparison (Bonferroni-corrected for multiple comparisons).

[c]Significant.

vidual variance in MOR stimulation-induced tracer displacement are not yet clear and need to be addressed in further studies.

Also, prior efforts to probe drug-induced presynaptic dopamine release in alcohol-dependent patients have shown blunted dopamine responses compared with control subjects (21,22). The contradiction between these results and our data is likely due to the use of different stimulus drugs. We tried to mimic the effects of ethanol-induced β-endorphin release by administering a specific MOR agonist (remifentanil). It is assumed, on the basis of preclinical data (27), that MOR agonist-initiated dopamine release is mediated by an increase in dopamine firing rather than an increase of dopamine synthesis. Instead, amphetamine—which was used by Martinez *et al.* (21) and Volkow *et al.* (22)—has been shown to stimulate dopamine release from newly synthesized cytosolic pools (45). Together, these findings indicate that a previously described reduced basal dopamine function in the striatum of drug-dependent individuals does not exclude an exaggerated phasic dopamine response to drug-specific stimulation.

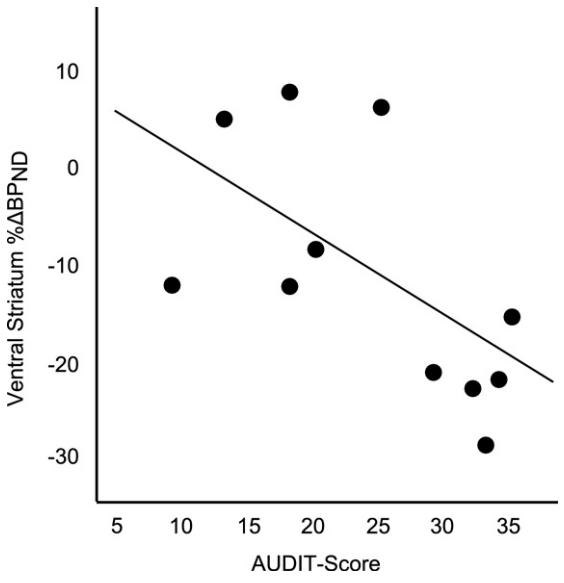

Figure 2. Correlation between self-reported dependence severity as assessed with the Alcohol Use Disorders Identification Test (AUDIT) and remifentanil-induced percentage change in nondisplaceable binding potential ($\%\Delta BP_{ND}$) of [^{18}F]fallypride in the ventral striatum of alcohol-dependent patients [$r(10) = -.62$, $p = .02$, and $r(8) = -.56$, $p = .046$, if age was included as covariate].

Several limitations of the study should be addressed. First, it needs to be noted that remifentanil is ultra-short-acting and has a fast clearance rate from the brain. In our study, remifentanil was administered as a single bolus injection, and as a consequence of methodological constraints (lack of arterial blood sampling), the data did not allow for an examination of changes in distribution volume after drug administration. Hence, although microdialysis showed an increase in dopamine in the rat brain after remifentanil administration (46), it is not clear whether the relatively long-lasting effects on tracer binding measured by PET can really be attributed to the short-lasting remifentanil effect. However, in previous studies, evidence has been provided for a temporal dissociation between agonist-induced dopamine increase as seen in microdialysis and a prolonged decrease in PET ligand binding (47). Specifically, extracellular dopamine increase after amphetamine challenge as assessed with microdialysis has been shown to last for 2 hours (48), whereas the in vivo decrease in PET binding potential lasts approximately 24 hours (49). This prolonged decrease in receptor binding compared with microdialysis findings has been attributed to agonist-induced D_2 receptor internalization (50). Hence, it seems well plausible that the observed changes in [^{18}F]fallypride binding are indeed a consequence of the remifentanil stimulation.

Second, for methodological reasons (to avoid the possibility that long-term effects of remifentanil would influence the baseline scan), scans were not counterbalanced across participants. Hence, it needs to be taken into consideration that differences between first and second scan might relate to repetition effects. However, test-retest variability for [^{18}F]fallypride was reported to be 3.8% in the striatum and 6%–8% in other limbic structures (51). The effects

found in our study were significantly larger, supporting the assumption that they could be attributed to remifentanil administration rather than repetition. Another drawback of our study is the lack of a placebo control condition. Because dopamine release has been reported during administration of placebo and related to its anticipated effects (52), the possibility needs to be taken into account that the effects observed in the present study might be triggered by psychological rather than pharmacological factors.

Third, we did not directly assess MOR density (or availability, respectively) in our subjects. Thus, it can only be assumed that elevated dopamine levels in heavy drinkers in response to remifentanil administration are a consequence of a MOR upregulation, suggested to exist in alcohol-dependent individuals (17).

Fourth, in the patient group, time of abstinence ranged between 8 and 126 days, although 10 of 11 patients had stopped drinking $<$50 days before participation in the study. Nevertheless, it cannot be ruled out that the mechanisms under study (opioid, GABA, or dopamine-related functionalities) were subject to change over the course of abstinence. To test this possibility, exploratory correlation analyses were performed, between days of abstinence and baseline BP_{ND}, days of abstinence and $\Delta\%BP_{ND}$, or days of abstinence and AUDIT score, none of which yielded significant results.

Finally, no effects of craving could be detected in our patient group. This is surprising and contradicts previous reports of significantly higher craving in detoxified alcohol-dependent individuals compared with control subjects. The most likely explanation for the lack of self-reported craving is that patients succumbed to social pressure. Because most of them had only recently stopped drinking and were still hospitalized, they might have felt obliged to negate an urge to drink alcohol.

To summarize, our data imply that direct stimulation of MORs enhances dopamine release in the brain reward system, providing important evidence for MOR-mediated control of the mesolimbic dopamine pathway. Our findings indicate that, in alcohol-dependent patients, sensitivity of the MOR-(GABA-)dopamine pathway is associated with dependence severity. After replication in larger samples, the results might help to specify pharmacological targets for the reduction of craving and prevention of relapse in detoxified alcohol patients.

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