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Brain levels of neuropeptides in human chronic methamphetamine users

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Summary

Animal data show that neuropeptide systems in the dopamine-rich brain areas of the striatum (caudate, putamen, and nucleus accumbens) are influenced by exposure to psychostimulants, suggesting that neuropeptides are involved in mediating aspects of behavioral responses to drugs of abuse. To establish in an exploratory study whether levels of neuropeptides are altered in brain of human methamphetamine users, we measured tissue concentrations of dynorphin, met-enkephalin, neuropeptide Y, neurotensin, and substance P in autopsied brains of 16 chronic methamphetamine users and 17 matched control subjects. As expected, levels of most neuropeptides were enriched in dopamine-linked brain regions such as the nucleus accumbens and striatum of normal human brain. In contrast to animal findings of increased neuropeptide levels following short-term methamphetamine exposure, striatal neuropeptide concentrations were either normal or moderately decreased in the methamphetamine users. In other examined dopamine-poor cortical and subcortical brain areas, neuropeptide levels were generally either normal or variably reduced. Although the neuropeptide differences might be explained by methamphetamine-induced damage to neuropeptide-containing neurons, our human data are consistent with the possibility that, at least in the human striatum, long-term methamphetamine exposure leads to an adaptive process that is distinct from that which increases neuropeptide levels after acute methamphetamine exposure.

Keywords

dynorphin; human; methamphetamine; neuropeptide Y; neurotensin; drug abuse

1. Introduction

The incidence of methamphetamine (METH) abuse is alarming, continues to be a societal problem and has been reported to significantly alter brain function. Specifically, cortical regions that are important for cognitive processing and functions such as decision making, complex memory, emotional status, auditory systems and visual systems are functionally altered in human METH-users (Chen et al., 2003; Gouzoulis-Mayfrank et al., 1999; London et al., 2005; Volkow et al., 2001). In fact, imaging studies incorporating positron emission

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tomography have demonstrated that dopamine (DA) transporters are reduced in the human striatum and these deficits are associated with poor performance in motor and memory tasks (Volkow et al., 2001). Thus, further investigation of the alterations in the brains of drug users could lead to a better understanding of the causes and consequences of METH abuse and perhaps help to develop more effective treatment strategies.

As such, animal data are highly suggestive that neuropeptide systems [dynorphin (DYN), met-enkephalin (MENK), neuropeptide Y (NPY), neurotensin (NT), and substance P (SP)] associated with basal ganglia and limbic DA pathways are influenced by stimulants because of the ability of these substances to alter activity of associated DA projections. For example, in experimental animal studies, short-term, high-dose METH treatments cause substantial increases in MENK, NT and SP levels in the DA-rich areas of the nucleus accumbens and caudate nucleus (Alburges et al., 2001; Letter et al., 1987; Wagstaff et al., 1996a) and these effects are mediated by drug-induced increases in dopaminergic activity. Using NT as an example, Wagstaff et al. (1996a) demonstrated that the DA D2 antagonist, eticlopride, blocked the METH-dependent NT increase in both the nucleus accumbens and caudate nucleus while the DA D1 antagonist, SCH 23390, only blocked the METH-evoked increase in NT levels in the caudate.

These neuropeptides have also been shown to alter activity of DA pathways known or suspected to influence motor functions, emotions, and addictive behavior [DYN (Reid et al., 1988; Steiner and Gerfen 1998); MENK (Steiner and Gerfen 1998); NPY (Midgley et al., 1994); NT (Wagstaff et al., 1996b); SP (Reid et al. 1988)]. Specifically, application of NT (Chapman et al., 1992) or SP (Tang et al., 1998) are reported to be excitatory, while DYN and MENK are reported to be inhibitory on striatal dopaminergic activity (Steiner and Gerfen, 1998). Consequently, there are actions both by drugs of abuse and these neuropeptide systems on DA pathways (Ervin et al., 1981; Steiner and Gerfen, 1998).

To date, no information is available on the status of the brain neuropeptide systems in humans exposed to psychostimulant drugs, apart from an investigation of preprodynorphin mRNA in cocaine users (Hurd and Herkenham, 1993). For this reason, we measured in an exploratory study the levels of five neuropeptides (DYN, MENK, NPY, NT, SP) considered to have the potential to interact with DA pathways in both DA-rich (striatum: caudate, putamen and nucleus accumbens) and DA-poor areas of autopsied brains of human chronic METH users (drug use verified by forensic drug analyses) and matched controls. We found that striatal neuropeptide concentrations in the METH users were, in contrast to those in the animal model studies, either normal or decreased.

2. Materials and methods

2.1. Subjects and brain material

Postmortem brains from a total of 17 controls (15 males and 2 females) and 16 chronic users of METH (11 males and 5 females) were obtained from medical examiner offices in the United States using a standardized protocol. Drug histories and other subject information have been previously reported (Wilson et al., 1996; Kalasinsky et al., 2001; Moszczynska et al., 2004). Dopamine levels in the caudate also have been reported previously for all of the METH cases (Wilson et al., 1996 and Moszczynska et al., 2004). This study was approved by Institutional Review Board of the Centre for Addiction and Mental Health at Toronto. There were no statistical differences in age (control, 32.7 ± 1.7 ; METH, 32.4 ± 2.0 years; mean $\pm$ SEM) or postmortem intervals (PMI, interval between death and freezing of the brain; control, 13.6 ± 1.6 hours; METH 15.3 ± 1.7 hours) between the control and METH users (Student's two-tailed *t*-tests). At autopsy, one half of each brain was fixed in formalin fixative for neuropathological analysis, whereas the other half was immediately frozen until dissection for neurochemical

analysis. Blood samples were obtained from all of the METH users and the control subjects for drug screening. Scalp hair samples for drug analyses were obtained from 14 of 17 controls and 12 of the 16 METH users. Levels of drugs of abuse in blood and other bodily fluids were measured by the local medical examiner whereas drug analyses in brain and hair samples were conducted at the Armed Forces Institute of Pathology (Washington, DC, USA). All METH users met the following selection criteria: (a) presence of METH on toxicology screens in blood (0.034 – 12.5 mg/l; Moszczynska et al., 2004; Wilson et al., 1996), autopsied brain (0.22 – 296 nmol/g tissue Moszczynska et al., 2004; Wilson et al., 1996), and, where available, sequential scalp hair samples (see Kalasinsky et al., 2004 for details on methods and assay sensitivity); (b) absence of any other drugs of abuse in these tissues (and absence of blood ethanol; Moszczynska et al., 2004); (c) evidence from the case records and structured interviews with medical examiner investigators, next of kin, and informants, of use of METH for at least 1 year before death; and (d) absence of neurological illness or brain pathology unrelated to use of the drug. The finding of METH (and metabolite amphetamine) in sequential hair samples in the absence of other psychostimulant and opiate drugs provides the strongest evidence that the subjects did not use these non-METH drugs of abuse in the recent (months) past. No significant abnormalities on neuropathological analysis were observed in autopsied brain of the METH users with the exception of gliosis in the putamen of one subject as reported in Moszczynska et al. (2004). However, quantitative neuronal cell counting analyses were not conducted. Examination of the case information disclosed no report of neurological illness (i.e., Parkinsonism) in any of METH users, although formal neurological testing was not conducted. All control subjects were neurologically normal and had no evidence of brain pathology on neuropathological examination. None of the controls had a history of drug use nor tested positive for drugs of abuse in blood or in autopsied brain. The control subjects were selected so that most had experienced, like the drug users, a sudden death. The suspected or known causes of death of the controls included drowning (n=1), leukemia (n=1), electrocution (n=1), accidental death/trauma (n=3), myocardial infarction (n=1), atherosclerotic cardiovascular disease (n=3), pulmonary embolism (n=2), valvular disease (n=1), hypertensive cardiovascular disease (n=1), massive cardiomegaly (n=1), compressional asphyxia (n=1), and slit throat (n=1). Details of the known or suspected causes of death of the METH users have been previously reported (Mirecki et al., 2004): acute METH intoxication (n=11), gunshot wound to the chest (n=2), coronary artery atherosclerosis and acute aortic dissection with METH intoxication as a possible contributing factor (n=3).

2.2. Measurement of Neuropeptides by Radioimmunoassay

The radioimmunoassay used to analyze the neuropeptide levels in this study was adapted from the methods described by Alburges et al. (2001). Tissue samples were homogenized in 0.01 mol/l HCl. The resulting homogenate was then placed in boiling water for 10 minutes in order to inactivate peptidases. Homogenates were centrifuged (17,000×g) for 30 min. Supernatant was then collected and an aliquot was used to determine the total protein for each tissue sample. Remaining sample was lyophilized overnight and stored at –80°C until the radioimmunoassay was performed. The concentrations of neuropeptides were determined with a modified solid-phase radioimmunoassay technique described previously (Alburges et al., 2001). Lyophilized samples were reconstituted in 300 µl phosphate-buffered saline (pH 7.4) containing 0.1% (w/v) gelatin and 0.1% (v/v) Triton X-100. Nunc-Immunoplates (ISC BioExpress, Kaysville, UT) were incubated overnight at 4°C with 40 µl of protein G solution (50 ng/100 µl in 0.1 mol/l sodium bicarbonate; pH 9.0). After washing the wells three times with wash buffer (0.15 mol/l K₂HPO₄, 0.02 mol/l NaH₂PO₄, 0.2 mmol/l ascorbic acid, 0.2% (v/v) Tween-20 and 0.1% (w/v) sodium azide; pH 7.5), 25 µl of a highly selective antiserum for one of the following were diluted in assay buffer (same as wash buffer containing 0.1% (w/v) gelatin): DYN diluted to 1:10,000; MENK diluted to 1:1,000; NPY diluted to 1:15,000; NT diluted to 1:20,000; SP diluted to 1:200,000. Following addition of DYN and MENK antisera, wells were incubated

overnight at room temperature. Wells for NPY, NT and SP assays were incubated with the respective antisera for 2 h at room temperature in order to allow the attachment of antibody to the protein G-coated surface. After incubation, wells were washed three times and 25 μ l of sample or standard(s) were added to each well and incubated for 2 h at room temperature. After incubation, 25 μ l of the labeled peptide (125 I-MENK, 125 I-NPY, 125 I-NT, or 125 I-SP) diluted with assay buffer to approximately 6500 dpm per 25 μ l, were added to the wells and incubated for 2 h at room temperature. Unlike the other labeled peptides, 125 I-DYN was added immediately after the samples (Hanson et al., 1988). After incubation, wells were washed, separated and placed in 12 $\times$ 75-mm polypropylene tubes and counted in a five-channel Packard Cobra II Auto-Gamma counter (Packard Instrument Co., Meriden, CT). The total and nonspecific binding were defined by adding 25 μ l of the labeled peptide to protein G-untreated and -treated wells, respectively. Quantities of neuropeptide immunoreactivity were determined by comparing bound to free [125 I]-DYN or -MENK in each sample to a standard curve (from 8 to 1000 pg/assay tube). For the quantities of immunoreactivity for [125 I]-NPY, -NT and -SP, bound to free comparisons were made using a standard curve from 1 to 125 pg/assay tube. The reproducibility of the assay was evaluated using cerebellum tissue spiked with 62 and 250 pg of each peptide. This technique has been demonstrated to be very reproducible, resulting in less than 10% variability between assays and less than 5% between sample and standard duplicates (Hanson et al., 1997).

2.3. Antisera

The MENK antiserum employed in this study was raised by Zymed Laboratories (South San Francisco, CA) using the single-point, site-directed peptide conjugation, antipeptide technique (Posnett et al., 1988). This highly selective MENK antibody displayed less than 1% cross-reactivity with leu-enkephalin or DYN and did not significantly cross-react with 1000-fold excess concentration of NT and has been used for other studies by this laboratory (Alburges et al. 2001). The antiserum to neuropeptide Y was prepared in our laboratory as described previously (Midgley et al., 1994). The DYN, NT and SP antisera were raised in New Zealand White rabbits as previously described (Hanson et al., 1988; Letter et al., 1987). These antisera recognize the DYN, NT or the SP carboxy terminus (respectively) and are highly selective, expressing no cross reactivity with 1000-fold excess concentrations of other endogenous neuropeptides such as DYN (for NT or SP antiserum), MENK, cholecystokinin, SP (for DYN or NT antiserum) or substance K.

2.4. Statistical Analyses

Correlations between neuropeptides and postmortem time for samples of controls and METH users were determined using the Pearson correlation test. Correlations between neuropeptides and brain drug levels of striatal or pallidal dopamine levels in METH users were also determined using the Spearman rank correlation test. As this was an exploratory study only, differences in neuropeptide levels (DYN, MENK, NPY, NT, SP) between the control and the METH groups were analyzed using the Student's Bonferroni's *t*-test. The criterion for statistical significance for all comparisons was $p < 0.05$.

3. Results

3.1. Selection of brain areas for examination

Subcortical brain areas were dissected using the atlas of Riley (Riley, 1943) whereas cerebral cortical brain areas were identified using Brodmann classification.

Initially, a number of regions of an autopsied brain from a neurologically normal control were analyzed for measurable levels of DYN, MENK, NPY, NT and SP. Based upon these preliminary assays, relevance to DA systems and availability, 10 human brain regions were

identified as having appreciable amounts of neuropeptides compared to cerebellum (a region mostly devoid of these neuropeptides) and were selected for further analysis in the METH users (See Table 1). These regions included the dopamine-rich striatal areas that have been examined in the animal studies mentioned in the Introduction (caudate, putamen, nucleus accumbens) as well as relatively dopamine-poor brain regions [frontal cortex (Brodmann area 9); occipital cortex (Brodmann area 18); temporal cortex (Brodmann area 22); parietal cortex (Brodmann area 39); thalamus (nucleus lateralis and medial pulvinar); and the substantia innominata].

3.2. Postmortem time and neuropeptide levels

The possible influence of postmortem time on neuropeptide levels was examined in all cases using the Pearson correlation test. Only one statistically significant correlation was observed in the control group: MENK in the medial pulvinar nucleus of the thalamus (-0.69 , $p < 0.01$).

3.3. Striatal dopamine vs. neuropeptide levels in the METH group

As METH is reported to damage caudate DA systems, Spearman rank correlations of DA levels in the caudate nucleus (reported previously in Wilson et al., 1996; Moszczynska et al., 2004) versus neuropeptide levels in the different brain areas of the METH users (current study) were performed in order to determine whether potential damage in the caudate dopaminergic system may be linked with alterations in neuropeptide levels in the human brains of METH abusers. When correlating DA levels with neuropeptide levels in the caudate, no significant correlation was found. When correlating DA levels in the caudate with neuropeptide levels in all other brain regions examined, a statistically significant correlation was limited to caudate DA and neuropeptide Y in the temporal cortex (Brodmann's area 22; -0.51 , $p = 0.044$) suggesting that the current results likely reflect alterations related to METH effects rather than its damage to the caudate DA system.

3.4. Brain methamphetamine vs. neuropeptide levels

Spearman rank correlations were performed in order to determine if there was a relationship between the amount of drug measured in the post mortem samples and peptide levels. Molar sum totals of METH (METH plus metabolite amphetamine) from occipital cortex were used for these determinations. For the METH users, statistically significant negative drug level correlations were only found between NT in the medial pulvinar thalamus region and total METH (-0.81 , $p < 0.001$).

3.5. Neuropeptide levels in METH users

In the striatum (Figure 1), statistically significant decreases in tissue levels (reported as percent of baseline) were observed for: MENK in the caudate (58 %), and putamen (53 %); NT (74%) and SP (66 %) in the caudate and DYN (56 %) in the nucleus accumbens. In the extra-striatal brain areas (Figure 2 and Figure 3), the different neuropeptide levels were either normal or moderately and variably decreased (range 27% to 84%) in all brain regions of the METH users with the exception of the Brodmann's cortical area 9 in which concentrations of MENK and SP were increased (156% and 132%, respectively). Brodmann's cortical area 22 was particularly influenced in the METH users and showed a decrease (48% to 84%) in concentrations of all measured neuropeptides.

4. Discussion

Until the present study, there has been no report as to the effect of heavy METH use by humans on CNS neuropeptide systems. All previous studies examining this issue were done in laboratory animals with METH being administered non-contingently under highly controlled

conditions (e.g. Frankel et al., 2005; Midgley et al., 1994; Reid et al., 1988; Steiner and Gerfen, 1998). Because of the very different circumstances, it is difficult to make direct comparisons between animal studies and the results from human METH abusers reported herein. For example, many of the humans included in the current study regularly abused METH for 10, 15 or 20+ years contrasting with the very short-term METH treatments used in the animals studies. The importance of such differences between the conditions associated with the animal experiments and this study are underscored by the observation that the pattern of neuropeptide responses in the METH abusers is very different from the findings in the short-term animal models. Consequently, it is important that future studies determine the basis for these differential neuropeptide responses in order to identify the role of these neuropeptide systems in the addiction process to METH.

Potential confounding variables

In order to help evaluate the significance of this study, we addressed a variety of confounding variables that might have influenced the results of our postmortem brain study. The brain regional pattern of neuropeptide levels showed the expected distribution with enrichment in the limbic nucleus accumbens brain region. The control and METH groups were matched with respect to age and postmortem time. Furthermore, little influence of postmortem time (up to 24 hours) on brain neuropeptide concentrations was observed. Like the METH users, most of the control subjects experienced a “sudden” death. To determine, as much as possible, whether any differences in the METH group could be attributed to the presence of the drug, forensic drug analyses were conducted in blood, brain, and, where available, hair of the drug users and controls to confirm use of METH and lack of use of other drugs that could be detected by these methods. From the analyses we found little correlation between drug levels at the time of death and changes in neuropeptide levels were found. Three of the METH-user population tested positive for opioids during the postmortem screening for drug and metabolites and these subjects were removed from the current study, (data not shown). However, the possibility cannot be absolutely discounted that the METH users might have taken other CNS active drugs or had other unevaluated characteristics that might have influenced neuropeptide levels. Despite these potential confounds, the standard error of the mean (SEM) reported in each figure argues that the relative difference between each individual subject within a group is reasonably tight.

Neuropeptide levels in normal human brain

In normal human brains we found a regionally heterogeneous distribution of the five neuropeptides examined, with four neuropeptides (DYN, NPY, NT and SP) highly concentrated in the nucleus accumbens (situated at the junction between the caudate and putamen) of the striatum and also in the anatomically close substantia innominata (just ventral to the anterior commissure; see plate T4-2066, Riley, 1943; Table 1). In most cases the absolute regional concentrations were consistent with what has been previously reported in human controls (Kleinman et al., 1985). In addition, absolute regional concentrations of the five neuropeptides were generally consistent with those reported in laboratory animals. Thus, concentrations of all of the neuropeptides in the cortical regions, the caudate nucleus, the putamen and the nucleus accumbens were very similar to the absolute amounts of these same neuropeptides found in corresponding structures in the rat [see DYN (Johnson et al., 1991); MENK (Alburges et al., 2001); NPY (Westwood and Hanson, 1999); NT (Wagstaff et al., 1996a) and SP (Hanson et al., 2002)]. Taken together, these results suggest that many of the neuropeptide systems in rats and humans are similar.

Striatal neuropeptide changes in METH users

Although no human data are available, in rodents there is a considerable literature demonstrating the sensitivity of these neuropeptides to METH administration. For example, in experimental animal studies, short-term, high-dose METH treatments cause substantial increases in MENK, NT and SP levels in the DA-rich areas of the nucleus accumbens and caudate nucleus (Alburges et al., 2001; Letter et al., 1987; Wagstaff et al., 1996a). These findings can be at least partially explained by an accelerated synthesis of neuropeptides (e.g. Adams et al. 2001). However, in contrast to the animal results, the concentrations of these neuropeptides in related brain regions were either not significantly different from controls or decreased in the human users of METH who all had chronic (at least one year) exposure to the drug. Assuming that the animal data are relevant to the human (see above), this suggests as one possibility that long-term METH use leads to an adaptive process that normalizes or down-regulates neuropeptide synthesis typically activated following short-term exposure. This possibility is supported by the observation of a general lack of correlation between brain METH levels and neuropeptide concentrations. Also consistent with this notion, neuropeptide changes or lack thereof could be due to the consequences of long-term, and not the acute, direct exposure to METH.

It is noteworthy that most (although not all) of the significant changes in neuropeptide tissue concentrations were decreases. This similarity in response pattern within a given brain structure (e.g. caudate) may be due to co-localization of neuropeptides within the same neurons. In support of this possibility in the caudate nucleus, NT and SP are co-localized in striatonigral neurons while NT and MENK are co-localized in striatopallidal neurons (Anderson and Reiner, 1990; Castel et al., 1993) and all three neuropeptides are similarly reduced in METH users (Figure 1). An argument against co-localization as a contributing factor to the patterns of neuropeptide responses is that DYN is also found in striatonigral neurons with NT and SP (Anderson and Reiner, 1990; Castel et al., 1993), but not only is not reduced in the caudate of METH users, but might actually be elevated (Figure 1). The role of co-localization in the response patterns of neuropeptides in other brain regions, such as Brodmann's cortical area 22, is more difficult to assess because little is known about the function or anatomy of these peptide systems.

Neuropeptide changes in dopamine-poor brain areas of METH users

In our exploratory study we also examined the effect of long-term METH use on neuropeptides in a sampling of cerebral cortical and subcortical brain areas that are comparatively (vs. striatum) poor in dopamine. A variety of changes, mostly decreases, were observed in each examined brain area with the exception of the nucleus lateralis of the thalamus (no significant differences) and temporal cortex. In principle, these changes could be related to reversible METH-induced changes in neuropeptide synthesis or turnover or to damage to neuropeptide-containing neurons or synthetic apparatus.

In Brodmann's cortical area 22 we found that levels of all five neuropeptides were decreased (48% to 84% of baseline), suggesting the possibility of non-specific damage to this brain region that was associated with drug use. However, this is unlikely as in our previous studies mean concentrations of other examined neurochemicals (G proteins, McLeman et al., 2000; choline acetyltransferase [ChAT], Kish et al. 1999) were normal in this cortical region as well as in other examined cerebral cortical brain regions of 12 of the 16 METH users examined in the current study. Although we did find previously in our study of the cholinergic synthetic enzyme, ChAT, that two METH users who had very high brain/blood METH concentrations also had a regionally widespread depletion of the enzyme (which might be explained by METH-induced hyperthermic damage to the enzyme protein), brain neuropeptide levels in these two cases were within the range of other METH users. An additional possibility is that the

neuropeptide changes observed in the postmortem cortical tissues in METH-users reflect altered cortical functions (Kalivas and Volkow, 2005; Winder et al., 2002). Consistent with this possibility, we observed that the neuropeptide levels in the cortical regions found to be particularly altered in the drug users were Brodmann's cortical areas 9 (frontal cortex), 18, 22 (temporal) and 39. These brain areas are especially important for functions such as decision making, cognitive processing, complex memory, initiative, planning, emotional status, auditory systems, and visual systems (Mai et al., 2003) and have been reported to be functionally altered in human METH-users (Chen et al., 2003; Gouzoulis-Mayfrank et al., 1999; London et al., 2005).

Biological relevance of neuropeptide changes

The results of our exploratory investigation must be considered preliminary and require replication. At a minimum, however, our findings suggest the need for animal studies of brain neuropeptides that more closely resemble the human experience and employ long-term administration of drug to establish whether any of the neuropeptide changes discovered in brain of human chronic METH users are also present in an animal model that more closely mimics the chronic condition present in typical METH addiction. For example, previous studies using rats employed non-contingent methods of administration and are thereby limited in terms of comparison. It is possible that neuropeptide systems respond differently in laboratory animals that contingently self-administer METH.

Assessment of the possible overall biological relevance of the neuropeptide changes is complicated by a lack of understanding regarding the functional significance of the neuropeptides in the examined extra-striatal brain regions (e.g., occipital cortex). Some of these regions, like substantia innominata, are not brain regions where rodent or human neuropeptide systems have been previously investigated making it impossible to compare our findings with other studies. However, extrapyramidal DYN and NT systems have been linked to drug abuse-related phenomena such as development and expression of psychostimulant sensitization (Rompere and Perron, 2000; Toyoshi et al., 1996) as well as the expression of addiction (Zachariou et al., 2006). Furthermore, direct infusion of DYN, MENK or NT directly into the brains of rodents blocks stimulant-evoked behavior (Castellani et al., 1982; Ervin et al., 1981; Ukai et al., 1992). Additional studies investigating basal neuropeptide levels in humans and animal models more appropriate to METH abuse in humans may help to define the specific role of the neuropeptide systems in these brain regions to METH-related phenomena.

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Abbreviations used

ChAT, choline acetyltransferase; DA, dopamine; DYN, dynorphin; MENK, met-enkephalin; METH, methamphetamine; NPY, neuropeptide Y; NT, neurotensin; SP, substance P.

Figure 1.

Neuropeptide levels in the human caudate nucleus, putamen and nucleus accumbens in control (no drug history) or METH abusers. Data ($n = 16-17$ per group) are expressed as percentage of baseline $\pm$ S.E.M. * $p < 0.05$ versus control. Abbreviations: dynorphin (DYN), metenkephalin (MENK), neuropeptide Y (NPY), neurotensin (NT), substance P (SP).

Figure 2.

Neuropeptide levels in the substantia innominata, medial pulvinar thalamus and lateral thalamus in control (no drug history) or METH abusers. Data ($n = 15-17$ per group) are expressed as percentage of baseline $\pm$ S.E.M. * $p < 0.05$ versus control. Abbreviations are the same as Figure 1.

Figure 3. Neuropeptide levels in the Brodmann's cortical area 9, 18, 22 and 39 in control (no drug history) or METH abusers. Data ($n = 15-17$ per group) are expressed as percentage of baseline $\pm$ S.E.M. * $p < 0.05$ versus control. Abbreviations are the same as Figure 1.

Table 1
Baseline neuropeptide levels (ng/mg) in 11 regions of control human brain samples (Mean $\pm$ SEM)

Brain Region	Dynorphin (DYN)	Met-enkephalin (MENK)	Neuropeptide Y (NPY)	Neurotensin (NT)	Substance P (SP)
Caudate Nucleus	0.42 $\pm$ 0.06	7.42 $\pm$ 1.14	4.83 $\pm$ 0.41	0.12 $\pm$.01	3.41 $\pm$ 0.31
Nucleus Accumbens	7.81 $\pm$ 1.13	12.83 $\pm$ 2.01	35.63 $\pm$ 2.52	1.16 $\pm$ 0.11	6.02 $\pm$ 0.42
Cortex 9	0.11 $\pm$ 0.01	1.54 $\pm$ 0.08	21.02 $\pm$ 3.30	0.07 $\pm$ 0.01	0.30 $\pm$ 0.02
Cortex 18	0.08 $\pm$ 0.01	1.02 $\pm$ 0.10	26.90 $\pm$ 1.67	0.08 $\pm$ 0.01	0.40 $\pm$ 0.03
Cortex 22 (Auditory Associative Cortex)	0.08 $\pm$ 0.01	12.14 $\pm$ 0.13	8.90 $\pm$ 0.55	0.033 $\pm$ 0.002	0.28 $\pm$ 0.02
Cortex 39 (Visual Cortex)	0.042 $\pm$ 0.003	7.12 $\pm$ 0.42	11.77 $\pm$ 0.78	0.081 $\pm$ 0.012	0.32 $\pm$ 0.025
Nucleus Basalis	1.27 $\pm$ 0.21	4.70 $\pm$ 0.61	15.56 $\pm$ 2.33	1.28 $\pm$ 0.26	3.30 $\pm$ 0.34
Nucleus Lateralis (Thalamus)	0.027 $\pm$ 0.002	1.17 $\pm$ 0.11	0.97 $\pm$ 0.068	0.101 $\pm$ 0.011	0.148 $\pm$ 0.03
Medial Pulvinar Thalamus	0.035 $\pm$ 0.004	0.355 $\pm$ 0.050	1.20 $\pm$ 0.15	0.224 $\pm$ 0.038	0.962 $\pm$ 0.200
Putamen	0.363 $\pm$ 0.067	16.48 $\pm$ 2.78	12.43 $\pm$ 1.71	0.165 $\pm$ 0.029	2.84 $\pm$ 0.39
Substantia Innominata (Extended Amygdala)	2.45 $\pm$ 0.77	6.46 $\pm$ 0.57	17.61 $\pm$ 2.06	0.78 $\pm$ 0.16	3.96 $\pm$ 0.53