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ORIGINAL INVESTIGATION

Effect of intranasally administered cholecystokinin on encoding of controlled and automatic memory processes

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Abstract
Rationale The neuropeptide cholecystokinin (CCK) is present in abundance in the central nervous system, where it is involved in the regulation of a wide range of functions. It also takes part in the modulation of memory processes, but its effect on human memory systems and processes is not yet well understood.
Objective The present experiment was conducted to examine the influence of CCK when present during encoding on later controlled and automatic recognition memory processes in humans.
Materials and methods A version of the process dissociation procedure was used to separate the contributions of controlled and automatic memory processes to participants' recognition memory performance. Data were analyzed within a multinomial modeling framework. Participants (N=64) received either 40 μg CCK-8S or placebo intranasally. The learning and test phases began 30 min after substance application. Behavioral, physiological, and self-report control variables were measured at three points of time during the experiment.
Results Compared to placebo, CCK increased the automatic, familiarity-based recognition memory component, while the parameter representing controlled, retrieval-based processes did not differ between groups. Also, in the exclusion condition of the test phase, the guessing parameter was reduced by CCK. None of the control variables were affected by the peptide.
Conclusions This result—the enhancement of the automatic recognition memory component when CCK is applied before encoding (and thus present during encoding and retrieval)—complements earlier results indicating that CCK decreases controlled, recollection-based recognition memory when applied during consolidation. The possible neuronal systems and processes mediating these effects are discussed.

Keywords Cholecystokinin · Intranasal · Memory · Controlled memory processes · Automatic memory processes · Process dissociation procedure

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Introduction

Cholecystokinin (CCK) is one of the most abundant and widely distributed neuropeptides in the central nervous system (Feldman et al. 1997). Among the different forms of CCK, its C-terminal sulfated octapeptide fragment CCK-8S is the most frequent in the CNS (Rehfeld and Nielsen 1995), interacting with the same affinity with both receptor subtypes, i.e., CCK_A receptors and CCK_B receptors (Moran et al. 1986; Noble and Roques 1999). CCK is involved in the regulation of a great variety of physiological and behavioral processes, such as satiety, analgesia, or anxiety (Crawley and Corwin 1994), but also of various memory-related functions. CCK and its receptors are found in high density in brain regions crucial for memory formation, among them most parts of the hippocampal formation, the cerebral cortex, and the amygdala (e.g., Lindefors et al. 1993; Lotstra and Vanderhaeghen 1987). The peptide's role in animal memory has been extensively studied (for review, see Itoh and Lal 1990; Rotzinger and Vaccarino 2003). In contrast, only a few studies have examined the impact of

CCK or its analogues on human memory. There were strong methodological differences between these studies, and the results were mixed. While Grasing et al. (1996) and Hommer et al. (1985) found no effect of the peptide on a variety of memory measures, there was an improvement in a measure of verbal memory in Pietrowsky et al. (1994) and a decrease in a measure of verbal memory in Shlik et al. (1998) after application of the peptide. Interestingly, in both of the Pietrowsky et al. (1994) and the Shlik et al. (1998) studies, the effect of the peptide was most pronounced in the recognition component of the involved memory tasks. It thus seemed obvious to analyze further which of the component processes that contribute to recognition judgments are affected by CCK.

Further studies with humans have evaluated the peptide's role on human information processing using evoked potentials (EP). It was repeatedly shown that application of CCK-8S leads to an increase of the P3-complex (Denecke et al. 2002, 2004; Pietrowsky et al. 1996, 2001). In these EP studies, CCK was administered intranasally, providing evidence for a direct nose–brain pathway for neuropeptides, possibly bypassing the blood–brain barrier (Born et al. 2002; Illum 2004; Pietrowsky et al. 1996).

Following these two lines of research with human participants, our research group extended the examination of intranasal CCK effects on human information processing to controlled (i.e., recollection-based) and automatic (i.e., familiarity-based) memory processes. This distinction is fundamental in recognition memory research (Kelley and Jacoby 2000; Yonelinas 2002). Controlled recollection is thought to demand larger processing resources and to be more susceptible to interference than automatic familiarity-based processes. Jacoby (1991) designed an experimental procedure and corresponding measurement model with the goal to assess separately the contributions of these two types of processes to observable recognition judgments. In the current study, we used Jacoby's procedure and an extension of his original measurement model.

Since its development, Jacoby's (1991) so-called *process dissociation procedure* has been used in a large number of studies, but none addressed the effect of neuropeptides on the processes underlying recognition judgments. Therefore, we examined the effect of CCK on the consolidation of controlled and automatic memory using the process dissociation procedure (Schneider et al. 2005). In this study, intranasal post-trial application of CCK decreased controlled recollection but not automatic, familiarity-based processes when compared to placebo. Thus, CCK decreased the involvement of controlled processes in recognition memory.

The present study was designed to extend our knowledge about CCK's effects to the processes during encoding, that is, when administered pre-trial. Coming from our first study, we expected an effect of CCK on controlled, recollection-based processes. No specific hypothesis concerning automatic, familiarity-based processes can be derived from the literature. As in our previous experiment, recognition judgments were analyzed because, from the studies on human memory conducted so far, it seemed that recognition is more sensitive to the peptide's effects than free recall.

Materials and methods

Participants

Participants were 64 (32 male, 32 female) nonsmoking healthy adults aged 26±4.64 years (range 20–39 years), who had been recruited via newspaper, the internet, or notices at the university. They had to be free of acute or chronic internal, neurologic, or psychiatric disease and of any medication, with the exception of oral contraceptives. Pregnant women were excluded from the study. Participants were instructed to sleep normally in the four nights before the experiment. Also, they had to fast overnight prior to the experimental session (i.e., to abstain from food or beverages except water for at least 12 h) in order to avoid endogenous CCK-secretion. All participants received 10 Euro for participation, and they gave written informed consent at the beginning of the experiment. The experimental protocol was approved by the local Ethics Committee in compliance with the declaration of Helsinki for human participants.

Psychological and physiological measures

Basic procedure and process dissociation measurement model

The basic procedure and the respective measurement model have been described in detail elsewhere (Jacoby 1991). Briefly, when applied to the analysis of recognition judgments, the experimental process dissociation procedure typically consists of three successive phases.[1] In phase 1, participants process a list of items, usually words, in a particular way. In phase 2, another item list with different processing instructions is presented. The subsequent recognition test in phase 3 includes the items from phases 1 and 2, as well as new items (distractor items). There are two test conditions. In the *inclusion* condition, participants are told

[1] The process dissociation procedure has been applied to all sorts of memory tests and also to attention and perception paradigms. The number of learning and test phases depends on the paradigm used. For a reference list on some earlier process dissociation studies, see Buchner et al. (1995).

to respond "old" to words presented in phases 1 and 2 and "new" to distractor items. In the *exclusion* condition, participants are told to respond "old" only to items presented in phase 2. Words from phase 1 and new words are to be called "new." On the basis of these instructions, the probability of calling a phase-1 item "old" in the inclusion task reflects the combined effects of controlled, recollection-based, and automatic, familiarity-based memory processes. In contrast, in the exclusion condition, an item presented in phase 1 would be called "old" only on the basis of automatic familiarity. Thus, no problems to follow the instructions suggest controlled memory processes; problems to follow the instructions suggest automatic, familiarity-based memory processing. From the "old" responses to items from phase 1 in both conditions, parameters for controlled recollection and automatic familiarity can be derived given a set of assumptions. As in Jacoby (1991), an independence variant of the process dissociation model was adopted (parameters representing recollection and familiarity were assumed to be stochastically independent).

The measurement model used in the current study, which was also used in our previous study (Schneider et al. 2005), was developed by Buchner et al. (1995). It is an extension of the original model by Jacoby that takes guessing and, hence, response bias into account (for further details and the model equations of the extended model, see Buchner et al. 1995).

Stimuli

The same stimulus material as in Schneider et al. (2005) was used in the present study (see this publication for a more detailed description of the process of word selection). Briefly, three word lists were matched with respect to imagery, concreteness, and word frequency based on German word norms by Baschek et al. (1977). Words were concrete five to seven letter nouns in singular form. Words with potentially "salient" characters (e.g., umlauts) were approximately equally distributed across the three lists. Words of one of the three lists were presented in phase 1, words of a second list were to be processed in phase 2, and words from a third list were added as distractor items in the recognition memory test in phase 3. The order of words within one list was randomized, as was the assignment of lists to the three phases.

Process dissociation procedure in the current study

In phase 1, participants read out loud the words presented one-by-one on a computer screen and decided as fast as possible whether they contained the letter "R" by pressing the "R" key on a keyboard. If not, participants were to press "P". In phase 2, which immediately followed phase 1, participants read out loud the words, determined how many letters it contained, and clicked on the corresponding number among a list on the screen as fast as possible. These tasks promote processing of the entire word but in a rather shallow fashion. The recognition phase was not mentioned in both phases to prevent rehearsal. Each word was shown for 5 sec in the middle of the screen, irrespective of the participants' reaction. The presentation of each word was separated by a two-second blank screen. In the subsequent recognition phase (phase 3), each test word was presented in the middle of the screen. The recognition judgment was made by clicking an "old" button or a "new" button below the word. No time limit was imposed on the recognition judgments. In the inclusion condition, participants were told to call a word "old" if they had looked for the "R" or counted the letters in it before and "new" if it was a new word. In contrast, participants in the exclusion condition were instructed to call a word "old" only if it they had counted the letters in it and "new" if they had looked for the "R" or if it was a new word.

Control variables

Auditory and visual vigilance tasks In order to standardize the events between CCK application and learning and also in order to control for possible effects of the peptide on attention, two computerized vigilance tests, both part of a well-established German test battery for attention (TAP, version 1.5; Zimmermann and Fimm 1999), were administered at two points during the experiment. Both tasks were nine minutes long and contained a high frequency of critical stimuli (i.e., stimuli participants had to respond to).

Blood pressure and heart rate Measures of blood pressure and heart rate were taken three times during the experiment, in order to ensure a similar physiological baseline for the participants prior to treatment and to control for possible effects of CCK administration during the study.

Self-report questionnaires Since CCK can influence self-perceived levels of activation (Pietrowsky et al. 1997) and anxiety ratings, we administered self-report questionnaires designed to measure these aspects. The German version of the shortened Adjective Checklist (AD-ACL; Imhof 1998) was used to measure self-reported levels of activation. It is designed to measure the two bipolar dimensions energetic arousal (Dimension A) and tense arousal (Dimension B). The German version of the State Trait Anxiety Inventory (STAI; Laux et al. 1981) was used to assess anxiety as a situation-dependent state (state scale, STAI-S) and as a personality trait (trait scale, STAI-T). The AD-ACL and the

STAI-S were given at three times during the experiment; the STAI-T was administered once at the beginning.

Design and procedure

The study was conducted as a 2 (substance: CCK vs. placebo) × 2 (test condition: inclusion condition vs. exclusion condition) factorial double-blind between-subject design. Each group consisted of 16 participants (eight male, eight female). The assignment to the different groups was randomized. A between-subjects manipulation of the test conditions was chosen in the process dissociation procedure because it has several advantages over a within-subject manipulation (Buchner et al. 1995).

Throughout the experiment, the sulfated form of the octapeptide was used (CCK-8S: H-Asp-Tyr[SO_3H]-Met-Gly-Trp-Met-Asp-Phe-NH_2, Calbiochem-Novabiochem, Schwalbach, Germany). CCK was dissolved in sterile water so that one puff of spray contained 10 μg CCK. Sterile water was also used as placebo. The phials containing CCK and placebo were kept in a deep freezer at −20°C and removed 15 min before each experimental session to defrost. During the experiment, participants received either 40 μg CCK or placebo intranasally. In the CCK conditions, a total of four puffs, each containing 10 μg CCK, was sprayed into both nostrils alternately, i.e., two puffs into each nostril. The placebo treatment was conducted analogously.

Experimental sessions took place either at 9:00 or 11.00 A.M. and lasted about 75 min. After having obtained informed consent, baseline measures of blood pressure and heart rate were recorded, followed by the STAI-T, STAI-S, and the AD-ACL. Immediately afterwards, the participants received either CCK or placebo. Next, they worked on the auditory and visual vigilance tasks. At the end of these tasks, blood pressure and heart rate were taken, and the STAI-S and AD-ACL were applied for the second time. Then, 30 min after application of the peptide, the three phases of the process dissociation procedure were conducted in direct succession. Finally, blood pressure and heart rate were measured again, the STAI-S and the AD-ACL self-reports were collected for the third time, followed by the second assessment of vigilance.

In all phases of the process dissociation procedure as well as during the attention tests, the instructions were presented on the computer screen for self-paced study. Participants worked on examples before each of the three phases of the process dissociation procedure and the vigilance tasks. Successful performance on the examples was a precondition for the continuation of the experiment. The process dissociation procedure as well as the vigilance tasks were presented via a 17-in. monitor and controlled by a personal computer. All participants were familiar with the handling of the mouse and the keyboard. During the entire experiment, participants sat in a sound-attenuated chamber in front of the computer screen.

Data analysis

For the analysis of the processes underlying the recognition judgments, we used a multinomial modeling approach (review in Batchelder and Riefer 1999). Multinomial models are stochastic models aimed at estimating the probabilities of latent (unobservable) processes on the basis of observed categorial behavior. This modeling framework provides appropriate techniques for computing confidence intervals of the estimates of the model parameters and for performing significance tests directly on these parameters. The model parameters representing controlled (c) and automatic (a) memory processes and guessing parameters for the inclusion (g_i) and the exclusion (g_e) condition, respectively, for both the CCK group and the placebo group were estimated from the participants' responses to phase 1 words and distractor words, resulting in a base model. Within the multinomial modeling framework, hypothesis testing can be accomplished by imposing restrictions on the base model that implement the hypothesis to be tested, for instance, by setting a certain parameter equal to another parameter. In the current study, such restrictions were imposed for the parameters c, a, g_i and g_e, i.e., each parameter of the CCK group was set equal to the respective parameter in the placebo group in succession. If CCK application prior to learning had an influence on any of the parameters, then forcing the parameters in both the CCK and the placebo groups to be equal should result in a significant misfit of the restricted model. After restricting the model, deviations of the expected category frequencies (which are a function of the newly estimated parameters after restriction) from the observed category frequencies were assessed by using a goodness-of-fit statistic; in this case, the approximately χ^2-distributed log-likelihood ratio statistic G^2. P values$\leq$0.05 were considered significant. The multinomial analyses were carried out using the AppleTree program (Rothkegel 1999).

Reaction times, number of correct responses, number of false responses, and misses in the visual and auditory vigilance tasks were analyzed using repeated measures ANOVA with one between-subjects factor (substance: CCK vs. placebo) and one within subject factor (time of measurement, two points of time). The same statistic was applied to the cardiovascular measures as well as STAI-S, AD-ACL-A, and AD-ACL-B, except that the factor time of measurement included three points of time. A t test for independent groups was conducted on the STAI-T. Analysis

of the vigilance data, the physiological and the self-report data were done using SPSS. *F* ratios were tested using the Greenhouse-Geisser procedure.

Results

Effects of CCK on recognition memory processes

The recognition data of one participant (inclusion, placebo) were completely excluded from analysis because data inspection suggested a problem in instruction comprehension. Also, two responses of another participant (placebo, exclusion) were excluded because very fast responses (<110 ms) indicated inadequate item processing. Figure 1 shows the estimates of the parameters reflecting controlled and automatic memory components as well as guessing processes in both the CCK and the placebo groups. The underlying frequency data are displayed in Table 1. As is typical of process dissociation procedure experiments, responses to items from phase 2 were not analyzed; they are presented here for reasons of completeness.

The parameters for controlled recollection hardly differed between the CCK and the placebo groups (CCK 0.35; placebo 0.31), and the restriction that $c_{CCK}=c_{placebo}$ did not result in a significant model misfit ($G^2(1)=0.39$, $p=0.53$). Interestingly, the parameter representing automatic, familiarity-based processes was clearly higher in the CCK than in the placebo group (CCK 0.71; placebo 0.60). The restriction that $a_{CCK}=a_{placebo}$ led to a significant model misfit ($G^2(1)=5.44$, $p=0.02$). Surprisingly, the parameter representing guessing processes in the exclusion condition

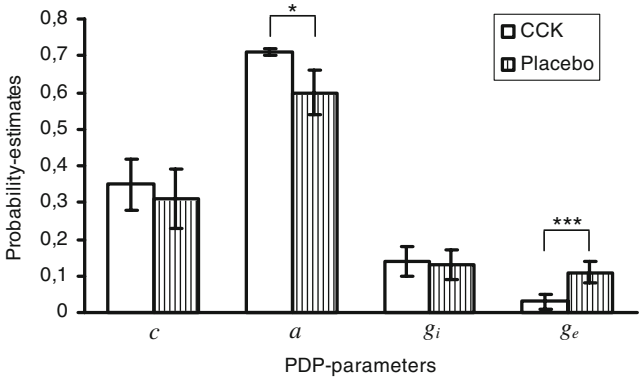

Fig. 1 Estimates for the parameters representing memory and guessing processes as a function of whether participants received CCK or placebo. *c* controlled recollection, *a* automatic, familiarity-based memory processes, g_e guessing "old" in exclusion condition, g_i guessing "old" in inclusion condition. The *error bars* depict the 0.95 confidence intervals. The *asterisks* indicate that parameters *a* and g_e in CCK participants differ from parameters *a* and g_e, respectively, in placebo participants. In other words, restricting these parameters to be equal (e.g., $a_{CCK}=a_{placebo}$) results in significant model misfits. *$p\leq 0.05$; ***$p\leq 0.001$

was much lower in the CCK than in the placebo group (CCK 0.03; placebo 0.11). The restriction that $g_{e\_CCK}=g_{e\_placebo}$ led to a significant model misfit ($G^2(1)=20.69$, $p<0.001$). There was almost no difference between the guessing parameters of both groups in the inclusion condition (CCK 0.14; placebo 0.13), and the restriction that $g_{e\_CCK}=g_{e\_placebo}$ did not result in a significant model misfit ($G^2(1)=0.15$, $p=0.70$).

Effects of CCK on control variables

For the analysis of all control variables, only the substance factor (CCK vs. placebo) was considered relevant.

Behavioral measures

The data of three placebo group participants in auditory and one placebo participant in visual vigilance had to be excluded from analysis because data inspection suggested problems with the identification of critical stimuli. In both tasks reaction times, number of correct responses, number of false responses as well as misses were analyzed.

Auditory vigilance Reaction times and number of false responses decreased from the first (t1) to the second (t2) point of measurement (reaction times, t1 493.46±74.04, t2 479.44±67.09; main effect $F(1,58)=6.15$, $p=0.02$, $\eta^2=0.10$; false responses, t1 1.12±1.28, t2 0.50±0.98; main effect $F(1,58)=14.37$, $p<0.001$, $\eta^2=0.20$). There was no main effect of substance (reaction times $F<1$; false responses $F(1,58)=1.10$, $p=0.30$, $\eta^2=0.02$) and no interaction between the two factors (reaction times $F<1$; false responses $F(1,58)=1.74$, $p=0.192$, $\eta^2=0.03$). The number of correct responses and the number of misses hardly changed over time (number of correct responses, t1 39.95±1.96, t2 39.72±1.86; number of misses, t1 0.62±1.25, t2 0.68±1.59). There was no main effect of time (correct responses $F(1,58)=1.23$, $p=0.27$, $\eta^2=0.02$; misses $F<1$), no main effect of substance and no interaction between these factors for these variables (all respective Fs<1).

Visual vigilance A similar pattern of results was observed for the visual vigilance task. Again, a decrease from the first to the second point of measurement was observed in reaction times (t1 459.58±110.38, t2 438.85±102.97; main effect $F(1,61)=7.88$, $p=0.007$, $\eta^2=0.11$) and number of false responses (t1 0.63±1.11, t2 0.32±0.62; main effect $F(1,61)=6.80$, $p=0.01$, $\eta^2=0.10$). No main effect of substance or an interaction between the two factors was observed for these variables (all Fs<1). As in auditory vigilance, the number of correct responses and misses remained quite constant over time (number of correct responses, t1 39.46±3.18, t2 39.56±3.07; number of

Table 1 Raw data for constructing the multinomial model: frequencies of "old" and "new" responses to words from Phase 1, from Phase 2, and to distractor words introduced in Phase 3

Condition	CCK						Placebo					
	Phase 1 words		Phase 2 words		Distractor words		Phase 1 words		Phase 2 words		Distractor words	
	"Old"	"New"	"Old"	"New"	"Old"	"New"	"Old"	"New"	"Old"	"New"	"Old"	"New"
Inclusion	267	53	273	47	45	275	229	71	261	39	39	261
Exclusion	149	171	213	107	8	312	142	177	195	125	36	283

misses, t1 1.33±2.49, t2 1.19±2.30), with no main effect of substance or time and no interaction between these factors (all respective Fs<1).

Physiological measures

Due to measurement problems during the experiment, the cardiovascular data of three participants in the CCK group had to be excluded from the statistical analysis.

The means of both the blood pressure (in mmHg) and heart rate (in bpm) control variables decreased over time in the CCK and the placebo groups, especially from the first to the second time of measurement. Statistical analysis revealed a main effect of time in all of these variables, that is, systolic blood pressure (t1 115.02±14.02, t2 110.72±13.64, t3 110.76±13.24; $F(2,118)=27.59$, $p<0.001$, $\varepsilon=0.92$, $\eta^2=0.32$), diastolic blood pressure (t1 72.20±9.65, t2 69.07±8.62, t3 69.37±9.77; $F(2,118)=11.37$, $p<0.001$, $\varepsilon=0.99$, $\eta^2=0.16$), and heart rate (t1 68.70±11.32, t2 67.58±10.53, t3 66.39±10.63; $F(2,118)=5.29$, $p<0.01$, $\varepsilon=0.95$, $\eta^2=0.08$). There was no main effect of substance in any of these variables (heart rate $F(1,59)=3.03$, $p=0.09$, $\varepsilon=0.95$, $\eta^2=0.08$, remaining Fs<1). Also, no significant substance × time of measurement interaction could be observed for these variables (all Fs<1).

Subjective measures

All scores represent the sum of the respective scale.

The STAI-T means in both groups did not differ much (CCK 36.31±7.24; placebo 35.44±7.36), and a t test showed that there was no significant substance effect ($t(62)=0.48$, $p=0.63$, $\eta^2<0.01$). In STAI-S, participants were comparable at baseline measurement. Values at t2 were slightly higher, then decreasing again in the placebo group while slightly increasing in the CCK group (CCK, t1 32.84±4.60; t2 33.09±5.49; t3 34.25±7.88; placebo, t1 32.97±5.41; t2 33.41±6.63; t3 32.06±5.41). There was no main effect of substance or time (both Fs<1), but a significant substance × time interaction ($F(2,124)=3.65$, $p=0.037$, $\varepsilon=0.83$, $\eta^2=0.06$), due to the difference between the groups at t3.

The AD-ACL-A ratings of one participant at t3 were not included because the test sheet was filled out incompletely. In both groups, ratings were comparable at each point of measurement, and there was a slight decrease in values from first to third measurement. There was a main effect of time (t1 32.08±4.37, t2 30.26±6.22, t3 29.45±5.85, $F(2,122)=11.06$, $p<0.001$, $\varepsilon=0.97$, $\eta^2=0.15$). No main effect of substance or a substance × time interaction was found (both Fs<1). Concerning the AD-ACL-B ratings, there was no main effect of time ($F(2,124)=1.04$, $p=0.34$, $\varepsilon=0.78$, $\eta^2=0.02$), no main effect of substance ($F<1$), and no interaction between these factors ($F<1$).

Discussion

The current study examined the effects of intranasal application of the neuropeptide CCK given prior to encoding and test on controlled, recollection-based and automatic, familiarity-based recognition memory processes. While CCK had no effect on controlled recollection, there was a significant increase of the probability of automatic, familiarity-based processes in the CCK group and also a substantial decrease of the guessing "old" probability in the CCK exclusion group. Only one control variable, namely STAI-S, was mildly affected by the peptide.

The results did not confirm our expectation of an effect on controlled memory processes when high levels of CCK are present during both encoding and retrieval. Taken together, the results from our previous study (Schneider et al. 2005) and the present results represent a double dissociation which might be explained by two complementary theoretical perspectives on memory functioning, one process oriented and the other systems oriented. The former view comprises the state dependency of memory observed in some studies (reviewed in Eich 1980, 1995). When applied to drugs, the main assumption is that retrieval of information about the target items is impaired when a subject's pharmacological state is changed between study and test sessions of an experiment, in comparison with a stable state in both occasions. In Schneider et al. (2005), the participants' pharmacological state was changed by CCK

(learning: without CCK; retrieval: with CCK) in comparison to the placebo group (both learning and retrieval without pharmacological active substance), resulting in an impairment of recollection-based controlled memory processes in the CCK group. In contrast, in the current study, encoding and retrieval were conducted in direct succession, and CCK (or placebo, respectively) was available in the brain at both instances. This state congruence might explain the absence of a CCK effect on recollection-based controlled memory processes. However, it does not account for the increase of the familiarity parameter, which may be better explained by the following system-oriented hypothesis.

Aggleton and Brown (1999; see also Brown and Aggleton 2001) suggested that controlled recollection depends primarily on a hippocampal–anterior thalamic axis, whereas automatic, familiarity-based processes rely on a distinct and independent system including the perirhinal cortex of the temporal lobe and the medial dorsal nucleus of the thalamus. It is possible that the enhancement of the familiarity parameter in the current study, in which CCK given prior to encoding and consolidation was minimized by design, is due to a stronger involvement of the perirhinal system. In contrast, in the study of Schneider et al. (2005), where CCK was given after encoding but prior to consolidation, the neuropeptide might have affected the hippocampal system primarily, leading to a reduction of controlled recollection. This interpretation is in line with the fact that consolidation of controlled, retrieval-based memory processes is heavily dependent on the hippocampus (Dudai 2004; McGaugh 2000).

CCK and its receptors are found in abundance in both the hippocampal and the perirhinal system (Innis et al. 1979; Lotstra and Vanderhaeghen 1987). Honda et al. (1993) reported a higher density of CCK_B receptors in the hippocampus in comparison to CCK_A receptors. There is some evidence for an impairment of memory functions after CCK_B receptor activation, while the CCK_A receptor has been associated with memory improvement (Lemaire et al. 1992, 1994a, b). Thus, the impairment in recollection in Schneider et al. (2005) might be attributed to predominant CCK_B receptor activation. It is possible that the increase in familiarity in the current study is due to higher CCK_A receptor involvement in the perirhinal system. However, to our knowledge, it is currently not known if this speculation is indeed paralleled by a respective receptor distribution.

One surprising result was the decrease of g_e in the CCK group. Thus, participants in the CCK exclusion group responded "new" to words that they could not recollect and that also did not appear familiar more often than participants in the placebo exclusion group. This suggests that CCK produced a more conservative response bias. However, why would this only hold in the exclusion condition? Subjectively, the exclusion condition is often experienced as being more difficult and demanding than the inclusion condition. Thus, only in this more difficult situation, the CCK group had a stronger preference for "new" responses than the placebo group. Given that neuropeptides are thought to act under neuronally activating conditions (Hökfelt 1991; Hökfelt et al. 2000) and assuming that the exclusion condition represents such an activating condition, the effect of CCK on non-recollected, unfamiliar items might become especially apparent here. However, we did not find a similar effect in our previous study (Schneider et al. 2005). Thus, at present, we are aware that this is a very speculative hypothesis implying many assumptions which need to be examined further.

Application of CCK did not have a strong impact on any of the control variables. Baseline levels in all control variables were quite comparable between the CCK and placebo groups. Over time, there was a decrease in all physiological control variables and in the AD-ACL-A, which probably reflects physiological adaptation to the testing situation. In both vigilance tasks, the reaction times as well as the number of misses decreased when measured for the second time, independent of group membership. This is most likely a practice effect that was not influenced by the peptide. The substance × time interaction in STAI-S values was due to the difference between both groups at the third time of measurement. We have no substantial explanation for this effect but do not consider it to be of major importance. First, the mean difference between both groups was only very small (about two points). Second, the scores in both groups before the realization of the process dissociation procedure, when CCK should have been available to central nervous structures, were quite comparable. Given the many tests on control variables, the result is probably due to chance.

We would like to stress three critical issues concerning the parameters that are derived from the process dissociation procedure. First, in pharmacological research, it is often quite difficult to find manipulations that increase automatic, familiarity-based processes as in the current study. Fillmore et al. (2001) and Mintzer et al. (2003) studied the effect of benzodiazepines on indices of the process dissociation procedure using a word-stem completion task. Both studies found increases of the automatic memory component after drug administration. However, Mintzer et al. have argued that the accumulating evidence on the effect of benzodiazepines contradicted a memory facilitation by the drug and that therefore the parameter estimates of the process dissociation procedure would not always be theoretically plausible. Second, there are concerns about the assumed relation between recollection and familiarity. Some authors (e.g., Curran and Hintzman 1995;

Joordens and Merikle 1993; Reingold and Toth 1996) have criticized Jacoby's assumption of independence between these two recognition components and argued in favor of alternative assumptions. Third, there is an ongoing discussion about the possible contamination of recollection and familiarity by response bias (i.e., guessing) which is also relevant for pharmacological research (e.g., Pompéia et al. 2003). The debate about these issues has not yet come to a closure. Concerning the use of the process dissociation procedure, other authors in benzodiazepine research point to the potential usefulness of this approach while acknowledging its methodological problems and suggesting alternative ways for data analysis (Hirshman et al. 2003; Pompéia et al. 2004). Regarding the relation between recollection and familiarity, Jacoby (1998) presented some evidence in favor of the independence assumption. Considering the problem of response bias, we would like to point out that in the current study, we made use of a measurement model (Buchner et al. 1995) for recognition memory that has been shown to be quite unaffected by response bias in comparison to Jacoby's (1991) original measurement model. Thus our main finding, the increase of automatic familiarity after CCK application, is very likely not a result of a contamination of the familiarity parameter by response bias; i.e., the influence of guessing on familiarity estimation is explicitly minimized by the measurement model.

In conjunction with the results from the Schneider et al. (2005) study, a differentiated pattern of CCKs effect on controlled and automatic memory processes emerges. With CCK present during consolidation, controlled recollection is decreased, but automatic memory processes are facilitated with CCK present during encoding. This double dissociation may be explained by the state-dependent nature of memory after drug administration or the involvement of different neuronal structures. Future studies should first aim to replicate and extend these findings, preferentially by using paradigms that refer to different two-process models of recognition memory. Second, to further test the system-oriented explanation, more specific CCK agonists or antagonists (Harranz 2003) should be applied to differentially affect the two recognition components at different stages of memory processing. It is possible that substances specifically activating or blocking CCK_B receptors primarily influence recollection, while substances predominantly acting via CCK_A receptors mainly influence familiarity. Third, CCKs' effect on other memory domains should be investigated. Among these, two are of special interest, spatial memory and emotional memory (declarative as well as non-declarative). The study of these memory domains would ensure better comparability of human memory experiments to the relevant animal studies in the field. Also, the latter would provide a link to the peptide's well-documented role in affect and motivation.

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