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Research report

Regional brain cholecystokinin changes as a function of friendly and aggressive social interactions in rats

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Abstract

Cholecystokinin (CCK) is the most abundant neuropeptide in the mammalian brain, and has been implicated in the regulation of a diversity of emotions and motivations including negative affect and stress responses. In this experiment, we assayed levels of CCK (CCK4/5 and CCK8) from tissue homogenates in intruder animals 6 h after resident–intruder inter-male aggression. Intruder animals that demonstrated submissive behavior (freezing and 22-kHz ultrasonic vocalizations) had higher levels of CCK in the tegmentum and posterior cortex as compared to non-submissive (i.e., "Friendly") intruder animals. Ultrasonic vocalizations (22-kHz) were positively correlated with CCK levels in the tegmentum, posterior cortex and pituitary. These data suggest that CCK may play a role in the generation of negative affective states indexed by 22-kHz ultrasonic calls in certain regions of the brain.
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Theme: Neural Basis of Behavior
Topic: Motivation and emotion

Keywords: Cholecystokinin; Aggression; Dominance; Neurochemistry; Social emotion; Social loss; Ultrasonic vocalization; Rat

1. Introduction

Cholecystokinin (CCK) is one of the most abundant neuropeptides in the mammalian brain. It modulates a diversity of physiological and behavioral processes [5], including feeding, pain, attention, sexual behavior, learning and memory, thermoregulation, and most prominently, aspects of anxiety [17,30,34,39]. This remarkably widespread neuromodulator operates through several ligands (e.g., CCK8 sulfated) and receptors (CCK1 and CCK2), which have multiple functions in the brain and body. In brain regions such as mesolimbic/mesocortical pathways, where CCK is co-localized with dopamine [24], it may promote motivation and attention [29] and thereby modulate preference for various rewards, ranging from food and drugs of abuse [11,56] to sexual feelings [12,33]. In other parts of the brain, perhaps via different receptor sub-types, it facilitates a variety of aversive anxiety-like emotional responses and associated memories [16–18].

Distinct motivational effects of CCK may be mediated by unique receptors of the CCK1 (formerly CCKA) and CCK2 (formerly CCKB) varieties [54]. Generally, facilitation of CCK1 receptor activity in the brain can promote attentional and appetitive effects such as male sexual arousal [12,33]. On the other hand, facilitation of CCK2 activity rather consistently provokes anxiety-like states in a large variety of paradigms [16]. Likewise, facilitation of CCK1-induced neural transmission improves social memories, while facilitation of CCK2 transmission retards social reminis-

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cence in adults [30,31]. However, blocking either receptor tends to facilitate maternal-odor induced place preferences in infant rats [45], which may reflect facilitation of pro-social, emotional regulatory processes [36,52], but it is known that early social bonding can be promoted by both positive and negative emotional states.

In many parts of the brain, CCK clearly facilitates a diversity of negative anxiogenic-type emotional states [10]. Negative affect may also be generated by peripheral CCK, which can yield conditioned place aversions [49]. It is also well established that infusion of CCK fragments, especially CCK 4/5 (e.g., pentagastrin) can facilitate panic attacks in humans [42,46] and anxious behavior patterns in animals [16,17]. Also, CCK release and gene-expression can be elevated in various brain areas by stressful events [15,19,20]. Unfortunately, efficacy of CCK receptor antagonists for the treatment of human anxiety remains to be clearly demonstrated [16], but this may be because the right kinds of anxiety in the right contexts remain to be evaluated [39].

In animal models, CCK2 antagonists are more effective in providing prophylaxis against the consolidation of the long-term anxiogenic consequences of stress (e.g., exposure to a cat) than the reversal of chronic anxiety states that have already developed [2]. It is also known that various stressors can modify CCK concentrations and release in several parts of the brain [41,47]. CCK release has been observed in the cortex during anxiety such as when animals are anticipating a confrontation with a more aggressive animal [4]. Likewise, trait anxiety in animals is related to levels of CCK2 receptor binding in the fear circuits of the brain [55], and placement of CCK into such areas facilitates fear responses, as in the amygdala [14] and periaqueductal gray (PAG) [25,35]. However, rage-type defensive behaviors are also sensitized by CCK in the PAG [32]. In sum, there is abundant pharmacological data that infusions of CCK receptor agonists can promote anxious and defensive behavior patterns, and these effects are mediated largely by CCK2 receptors. Such central CCK changes may provide long-term reinforcement and solidification of stress–anxiety states in the nervous system [1,7].

One of the intriguing aspects of CCK-induced anxiety is that the effects are dependent on environmental contexts, being evident in some, but not all behavioral paradigms [18]. In terms of the actual release of CCK in the brain, the most robust effect has been seen in animals anticipating social defeat [4]. Accordingly, the aim of the present research was to determine how CCK levels vary as a function of adult social interactions that might be characterized as friendly or antagonistic using a resident–intruder paradigm in individually housed adult male rats. The approach was correlative. We monitored CCK changes in 14 regions of the brain as a function of either positive or negative social interactions. The work was conducted to help estimate in which brain areas CCK changes may be provoking long-term changes in anxiety-type states, and also provide information about how CCK dynamic changes in various brain regions relate to each other and to various behaviors which are evident in situations characterized by social defensiveness or "friendliness". Individually housed male rats were exposed to half hour social encounters using a resident–intruder paradigm that often leads to aggressive episodes in some animals and comparatively friendly (i.e., non-aggressive) interactions in others. In this study, we evaluated regional CCK brain changes in these two types of animals. To estimate what kinds of affective states were accompanying these social interactions, 22- and 55-kHz ultrasonic vocalizations were monitored, because there is abundant evidence that they reflect negative and positive affect states, respectively [28,38,40].

No specific a priori hypotheses were advanced. The study was envisioned as an exploratory study to evaluate the extent to which this stress-responsive system adjusted to social stress. Specific hypotheses could have been generated from prior studies evaluating release of CCK during stress [4,41] but since little is known about how release patterns are compensated by de novo synthesis patterns, any specific hypotheses would have been premature. For now, our main interest was to see if there were any global or regional shifts in brain CCK levels, which might suggest how this brain system responds to competitive social stress that can be deemed to be aversive to rats.

2. Materials and methods

2.1. Housing

Neurochemical data reported in this study were derived from 24 adult Male Long Evens rats (age ~5 months), born and raised in the Bowling Green State University Animal facility—10 animals were controls that experienced no aggression, and 14 were animals that were exposed to social encounters, from which a sub-set of 6 animals were selected that clearly exhibited submissive, defeat types of behavior (designated as the "Submissive" group), whereas the other 6 were not attacked, and the social interaction appeared unaggressive (designated as the "Friendly" group). To evoke aggression, we used six pre-screened "resident" males that had, during prior screening, exhibited moderate aggression toward most intruders, and six "residents" that were generally "friendly" or at least non-aggressive toward intruders. Since two of the 14 intruders exhibited strong aggression toward the residents, they were excluded from the "Submissive/Friendly" comparisons, but their neurochemical results were included in the overall exploratory social vs. nonsocial comparisons (Fig. 3).

Since weaning (at 21 days of age), all test animals had been housed individually in 20×40×20 cm translucent polycarbonate cages with corncob bedding, with continuous free access to food and water. Temperature was maintained

constant, at about 21 °C and lighting was on a 12:12 light/dark cycle with lights on at 8:00 am. All behavioral testing occurred during the light part of the cycle.

2.2. Behavioral testing

At the beginning of resident intruder testing, all animals, both residents and intruders, had been isolate housed for over 3 months. All testing was conducted in a separate test room, so that the social activities would not be disruptive to the rest of the colony. Social aggression testing consisted of placing the intruder animal into the home-cage of the resident animal for 30 min under dim (~2 lx) light. A plastic lid was placed on top of the residents home cage with a small hole (6×6.5 cm) to position the ultrasonic sound sensitive microphone. Control animals were treated in the same way, except for the aggression testing: Half the controls were placed into the testing room alone in their home-cage (own cage controls n=5) while half were placed into the empty cage of a conspecific male (other cage control n=5). Since there were no neurochemical differences in these groups, their data have been combined into a composite control group (n=10).

Social interactions of the experimental animals were archived with a DVD recorder, for subsequent scoring of social behaviors. During these recordings, ultrasonic vocalizations (USVs) were also monitored using a Petterssen D980 ultrasonic detector (Uppsala, Sweden). Both the frequency division (1/10 division) and heterodyne channel (tuned to 55 kHz, range ±5 kHz) were recorded onto DVD via separate audio channels.

2.3. Behavioral coding

All visually evident behavioral data was analyzed blindly and independently of knowledge of the biochemical data. All ultrasonic vocalizations were also scored by individuals who were blind to experimental conditions. Videotape records were hand scored for three behaviors: (1) dorsal contacts, (2) bites (most of which were accompanied by audible squealing by the intruder), and (3) the subsequent freezing behavior. A freezing bout was counted after ~5 consecutive seconds without movement (except sniffing). Total duration of freezing was used for subsequent analyses. Dorsal contacts consisted of one animal having both its front paws on the dorsal surface of the other animal, this behavior was scored as frequency of occurrence for each animal, as was biting. Other behaviors that commonly occur during the more serious forms of this type of aggression (i.e., sustained dominance on-top and submissive supine postures) did not occur frequently with the comparatively mild aggression observed in these animals. Ultrasonic vocalizations were hand scored from sonographic displays, with 20-kHz USVs scored from the frequency division channel, while the 55-kHz ultrasonic vocalizations scored off the heterodyne channel tuned to that wavelength.

To summarize, of the total set of 14 tested intruder animals, those that had received at least one bite were placed into the "Submissive" group (n=6). Among this set, there was one pair where both animals did receive a bite, but the intruder clearly received more bites, and initiated freezing, and hence was assigned to the submissive group. The animals that had not given or received any bites during the test period were placed into the "Friendly" group (n=6).

2.4. Brain tissue sampling

Six hours after testing, rats were rendered unconscious with ambient carbon dioxide, decapitated and brains as well as pituitary (abbreviation *Pit*) were extracted. From the extracted brains the following brain areas (with abbreviations) were dissected: *Hyp*—Hypothalamus; *FCTX*—frontal neo-cortex (consisting of the frontal pole just anterior to the caudate-putamen); *CTX1*—anterior neo-cortex over basal ganglia; *CPU*—caudate-putamen; *Sept*—Septal area including some Bed nucleus of the stria terminalis and fornix; *BF*—all basal forebrain ventral to the CPU including N. Accumbens; *Thal*—the entire thalamus; *Amyg*—amygdala and surrounding periamygdala and temporal cortex; *CTX2*—posterior neo-cortex surrounding the body of the hippocampus; *Hipp*—body of the hippocampus, excluding dorsal area right above the thalamus; *SN/VTA*—Substantia Nigra and Ventral Tegmental Area: *Tect*—Tectum including colliculi, periaqueductal gray and surrounding mesencephalic tissue including some posterior thalamus and hypothalamus; *Teg*—tegmentum consisting of the ventral midbrain above SN-VTA and some pontine tissue. Gross separation of areas was first achieved by obtaining approximately 2–3 mm coronal sections with a calibrated tissue block, and subsequent fine dissection was conducted, on ice-cold platform, using microdissection tools. Tissue samples were promptly placed in eppendorf tubes and frozen on dry ice. Samples were stored at −80 °C until assayed. In statistical comparisons, degrees of freedom may vary slightly due to loss of a few brain samples during processing. When inter-correlations were done between CCK levels, Pearson product-moment correlations were calculated, but for relationships where the data were not normally distributed, non-parametric Spearman's correlations were employed.

2.5. CCK assays

Biochemical procedures were essentially the same as previously described [6]. The brain samples were homogenized in 0.1 N HCl, an aliquot was taken for protein-determination (Bradford Method) and another for radioimmunoassays of CCK. The utilized method detects mainly CCK8, including both sulfated and unsulfated varieties, with a moderate preference for the sulfated form. It has little affinity for CCK 4 or 5, of which there is, in fact, very little

in rat brain. On half the animal brain tissues, two separate assays were conducted, and the test–retest reliability of the assay was r=0.92 across the replicate samples.

3. Results

3.1. Behavioral

The Submissive animals typically received one bite about half way into the test session, and thereupon they exhibited clear and significant freezing behavior as compared to the Friendly pairs ($t(10)$=3.96, P<0.005; Fig. 1). There was also a non-significant trend for the Submissive animals to exhibit fewer dorsal contacts than Friendly ones ($t(10)$=2.07, P<0.10). Inter-observer reliability scores for Dorsal Contacts, Freezing and Bites were 0.87, 0.99, and 0.98, respectively (Pearson correlations, all p's<0.05).

Behavioral asymmetries were also evident in the emitted vocalizations. Resident–intruder pairs that produced Submissive animals exhibited significantly more 22-kHz USVs during testing ($t(10)$=2.45, P<0.05) and a non-significant trend for less 55-kHz USVs ($t(10)$=1.94, P<0.10; Fig. 2). Without devocalizing one animal, it is impossible to unambiguously say which animal is doing the vocalizing, but based on previous data [50], as well as our observation of thoracic movement, it can be reasonably confidently concluded that most of the 22 kHz USVs were emitted by the submissive animal. Indeed, such vocalizations always started after the bite: There were no recorded 22 kHz vocalizations during the 5 min prior to the bite. On average, the first 22 kHz vocalization occurred 155 s after that bite, and there were 111.3 USVs/5 min during the subsequent 5 min (Wilcoxon Signed Ranks Test $Z(5)$=2.20, P<0.05). In contrast, practically all the 50 kHz vocalizations in the Submissive encounters occurred prior

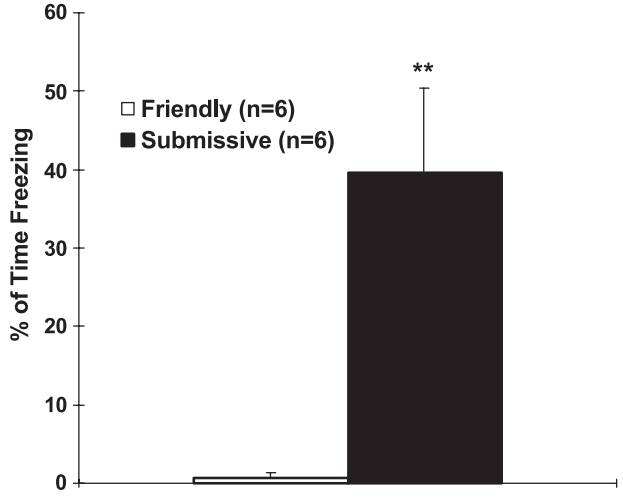

Fig. 1. Mean±S.E.M. time spent freezing during 30 min resident intruder encounter for animals in the Submissive and Friendly groups. **P<0.01 (between subjects two-tailed t-test).

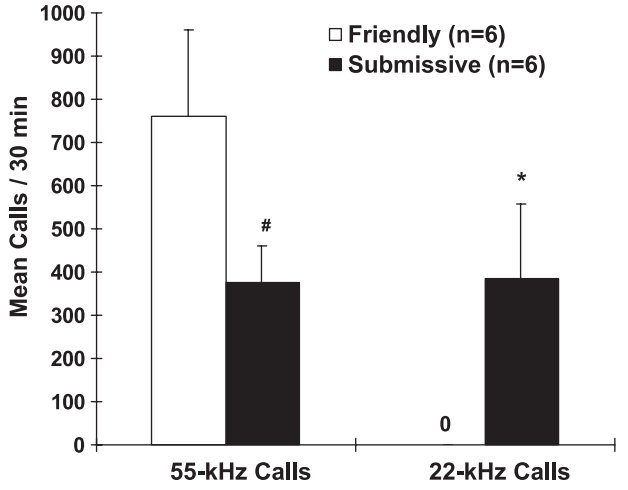

Fig. 2. Mean±S.E.M. ultrasonic vocalizations during 30 min resident intruder encounter for animals in the Submissive and Friendly groups. #P<0.10, *P<0.05 (between subjects two-tailed t-test).

to the bite, but we cannot specify which animals were exhibiting those USVs. Finally, we measured the peak frequencies of the first 10 calls per pair of both the 20- and 50-kHz call types from our frequency division recordings. The mean (S.E.M.) peak frequency for the 50-kHz calls was 51.9 (0.6) and 22.5 (0.3) kHz for the so-called 20-kHz calls.

3.2. Neurochemical

The overall levels of CCK in the various brain areas of all the animals, 10 controls and 14 animals tested socially, are summarized in Fig. 3. There were the expected massive differences in CCK levels between different brain regions [6], the variance of which precluded any meaningful overall ANOVAs for main effects and interactions for the test groups across all brain areas. However, to estimate for potential effects of the overall social variable, we do present paired group comparisons for individual brain areas, to highlight potential regional changes that may be of importance since they remained significant even with a Fisher LSD post hoc test to control for false positives. Frontal cortical CCK levels were lower in the socially tested animals (mean±S.E.M.: 3.54±0.32) as compared to isolate tested controls (4.67±0.42; $t(22)$=2.30, P<0.05). We would note that these differences need to be deemed provisional, especially since they are no longer significant once the two aggressive intruders are discarded that obviously needed to be dismissed from the Submissive/Friendly analysis.

Two brain areas exhibited significantly greater CCK levels for the Submissive and Friendly animals (Fig. 4): in CTX2, namely the posterior neocortical sample ($t(10)$=2.34, P<0.05) and the tegmental samples ($t(10)$=2.55, P<0.05), with a marginal trend in the same direction for the thalamus ($t(10)$=1.99, P<0.10). Since there were 14 planned comparisons (one for each of the 14

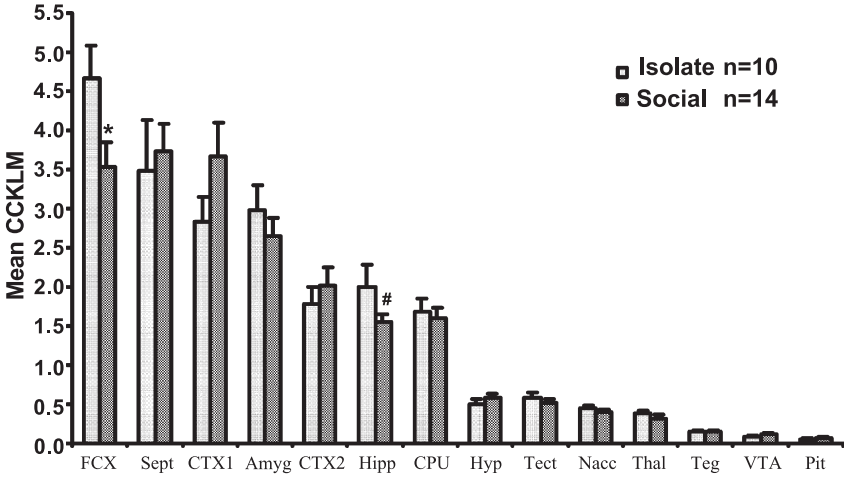

Fig. 3. Mean±S.E.M. concentration of CCK-8 various brain areas in control animals tested alone (isolate group) and animals tested in the resident intruder paradigm consisting of the animals in the Submissive and Friendly groups (social group). For list of abbreviations, see Section 2. *P<0.05, #P<0.10 (between subjects two-tailed t-tests).

brain regions tested), there is a reasonable possibility that one of the above changes is significant simply by chance. Indeed, calculating the probability of Family-Wise error for these neurochemical results yielded a 51% probability for each ANOVA and <5% for each comparison.

3.3. Neurochemical and behavioral intercorrelations

With regard to inter-correlations between CCK levels in the various sampled brain regions (all summarized in Table 1), there were two large clusters of intercorrelations: (1) The CPU positively correlated to thalamus, hypothalamus, amygdala, septal, basal forebrain, and frontal cortical areas as well as the neo-cortex (CTX1) overlying the CPU (r's=0.48 to 0.61, p's<0.05); (2) The CPU negatively correlated with the tegmentum (r=−0.41, P<0.05). (3) Of the areas positively correlated with the CPU, CTX1 was highly related to the hypothalamus, and thalamus, and neocortex was highly related to the thalamus and amygdala (r's=0.43 to 0.58, p's<0.05). Within this inter-related cluster, only the septal and hypothalamic CCK levels were strongly related (r=0.57, P<0.001). Totally separate from the above clustering, a singular positive correlation was evident between CCK levels in the basal forebrain sample (which included nucleus accumbens) and the substantia nigra/ventral tegmental area (r=0.53, P<0.01). These complex inter-relations suggest several independently related CCK control mechanisms in the brain. Of course, a certain number of the correlations in this and subsequent tables could have been expected to be significant by chance alone, but since this was an exploratory study, we share all results without further qualifications.

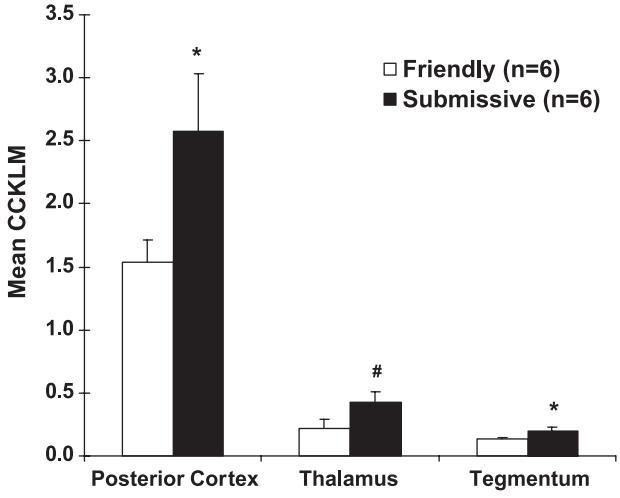

Fig. 4. Mean±S.E.M. concentration of CCK-8 various brain areas in Submissive and Friendly animals. *P<0.05, #P<0.10 (between subjects two-tailed t-tests).

Analysis of behavioral cross correlations (Table 2) yielded inter-relationships between 22 kHz USV and freezing (Spearman's r=0.84, p=0.001), which are both measures of negative emotionality, and between 50 kHz USVs and dorsal contacts (r=0.73, P<0.01), which have both been interpreted to be indicators of positive social interactions. In addition, 20-kHz calls were negatively correlated with dorsal contacts (Spearman's r=−0.65, P<0.05).

Relating brain CCK levels to behaviors also yielded a few relationships (Table 3). A significant correlations between behavior and regional CCK levels and emotional sounds was found between 22 kHz USVs and CCK levels of the posterior neo-cortex overlying the hippocampus (designated CTX2) as well as the pituitary (Spearman's r=0.70 and 0.60, p's<0.05). This functional relationship was reinforced by the presence of significant correlations between freezing behavior and CCK levels in CTX2 and pituitary (Spearman's r=0.69 and 0.62, p's<0.05). It is also noteworthy that CCK levels in this posterior neocortical region was only significantly correlated to levels of CCK in one other brain sample—the pituitary (r=0.47, P<0.05),

Table 1
Correlation matrix comparing CCK levels for each brain area sampled in all the animals tested (n=24)

	FCXT	CTX1	SEPT	AMYG	TECT	TEG	HIPP	HYP	THAL	PIT	CPU	CTX2	VTA
CTX1	−0.02												
SEPT	0.24	0.38											
AMYG	0.58**	−0.07	−0.02										
TECT	0.09	−0.36	−0.37	−0.15									
TEG	−0.08	0.06	0.03	−0.27	0.32								
HIPP	0.15	0.20	0.25	−0.19	−0.12	−0.15							
HYP	0.16	0.49*	0.57**	−0.01	−0.11	−0.15	0.48*						
THAL	0.43*	0.44*	0.38	0.24	−0.13	0.09	0.32	0.28					
PIT	0.01	−0.17	−0.24	−0.04	0.41*	0.44*	−0.14	−0.14	0.26				
CPU	0.49*	0.51*	0.60**	0.48*	−0.29	−0.41*	0.26	0.57**	0.61**	−0.21			
CTX2	0.32	−0.21	0.24	0.20	−0.03	0.12	0.16	0.20	0.16	0.47*	0.10		
VTA	−0.02	0.31	0.25	−0.08	0.13	0.12	0.13	0.40	0.24	0.36	0.27	0.28	
BF	0.37	0.24	0.26	0.17	0.14	−0.08	0.20	0.36	0.37	0.00	0.48*	−0.21	0.53**

For brain area abbreviations, see text.
* P<0.05 Pearson correlation (two-tailed).

which was also significantly related to CCK in both tectum and tegmentum (r's=0.41 and 0.44, p's<0.05).

4. Discussion

Socially isolated animals have a high tendency to exhibit aggressive behaviors when reunited with other animals, and residents typically tend to prevail over intruders. At the point where a fight clearly occurs, as highlighted by biting, the apparent affective quality of the interaction becomes negative, as can be monitored with the emission of 22 kHz vocalizations [40]. This is typically followed by the freezing of the animal that has been attacked, and in the present work, the two variables were significantly related. This suggests that to some degree, the two measures reflect a common underlying process, perhaps one characterized by a form of negative affect [28]. Indeed, the correlations between freezing and the 22 kHz USVs suggest that those vocalizations were largely emanating from the Submissive animals, and this is confirmed by the temporal analysis of the 22 kHz USVs with respect to the initial biting attack. When no aggression occurs, animals typically exhibit more 50 kHz USV, a sound that typically accompanies positive social interactions [27,38], but we have no empirical verification that these vocalizations came largely from the Friendly animals.

Table 2
Correlation matrix of the behavioral measurements recorded during the 30 min social encounter for animals in either the submissive or friendly group (total n=12)

	DC	Bites	Freeze	50 kHz
Bites	−0.15			
Freeze	−0.54	0.55		
55 kHz	0.73**	−0.19	−0.52	
20 kHz	−0.65*	0.19	0.84**	−0.39

Pearson correlations were used for all comparisons except comparisons including 20-kHz vocalizations, in which Spearman correlations were reported.
* P<0.05 (two-tailed).
** P<0.01 (two-tailed).

Of the many neurochemical changes implicated in the regulation of emotions [38], CCK systems have been linked to negative affective processes in many rodent [18] as well as a few human studies [42]. Previous work has observed the release of CCK in rats as a function of social defeat [4], and the present results are congruent with the view that social aggression can modify CCK dynamics. In our animals, the most robust finding was that CCK levels in posterior neo-cortex, specifically the areas lying over the body of the hippocampus, exhibit elevated CCK in social-intruder animals that became clearly submissive as a result of attack compared to animals that were not attacked (i.e., those that remained "friendly"). Similar trends were observed in thalamus and tegmentum, but because of the modest size of the effects, these patterns need to be replicated before any definitive conclusion can be reached.

The mere act of social interaction, by itself, did not have any robust *overall* effects on brain CCK (Fig. 3), but the marginal reductions in CCK levels in the frontal cortex, and perhaps to a lesser extent in the hippocampus deserve attention in any follow-up studies. Of course, considering the number of brain areas sampled and the modest magnitude of these effects, follow up studies are needed to verify the consistency of such trends.

Even if these results can be replicated in future work, it must be emphasized that measures of total peptide levels in brain sub-regions do not allow one to specify which aspects of the underlying biochemical dynamics are being affected; whether synthesis, release, and/or degradation. The changes only specify that the system is responsive to certain types of environmental events. Taken at face value, these results suggest that during social interactions, regardless of its quality, rats may be utilizing more CCK in frontal cortical regions than when they are alone. However, during

Table 3
Correlational comparison of the behavioral measurements recorded during the 30 min social encounter for animals in either the submissive or friendly group (total n=12) to CCK levels for each brain area sampled

	FCXT	CTX1	SEPT	AMYG	TECT	TEG	HIPP	HYP	THAL	PIT	CPU	CTX2	VTA	BF
DC	−0.22	0.09	−0.10	−0.01	0.06	−0.17	−0.16	−0.05	−0.23	−0.30	−0.03	−0.58*	−0.14	−0.07
FREEZE	0.06	−0.35	−0.04	−0.27	0.21	0.53	0.10	−0.23	0.25	0.62*	−0.39	0.69*	0.24	−0.10
50 kHz	−0.33	0.33	0.26	−0.24	−0.04	−0.08	−0.10	0.29	−0.29	−0.31	0.20	−0.53	0.24	0.29
20 kHz	0.31	−0.27	0.21	0.03	−0.24	0.48	0.33	0.16	0.54	0.60*	−0.14	0.70*	0.26	−0.09

Pearson correlations were used for all comparisons except comparisons including 20-kHz vocalizations, in which Spearman correlations were reported.
**P<0.01 (two-tailed).
* P<0.05 (two-tailed).

unfriendly interactions, there may have been an upregulation of CCK synthesis in posterior neocortical, tectal and to a lesser extent in the thalamic areas in Submissive animals. Of course, the differences could also have been due to elevated CCK release in the Friendly animals, and other possible changes in patterns of synthesis, use, and degradation are possible [3]. Thus, the present results can only be clearly interpreted once there is more data on the CCK metabolic dynamics, especially gene expression patterns of CCK and the relevant receptors in these brain regions as a function of various types of social interaction. Based on past data, it is reasonable to expect that severe social stress (i.e., defeat) not only recruits arousal of brain CCK systems, but may also facilitates CCK peptide and receptor gene transcription and/or translation in parts of the brain that mediate the aversive anxiogenic properties of CCK. At present, there is only modest evidence for such possibilities [51].

In the present data set, the strongest findings were evident in the correlative analysis between regional brain CCK levels and behavioral tendencies. The 22 kHz vocalizations in the 12 experimental animals (those summarized in Fig. 2) were strongly correlated with posterior neocortical CCK levels (r=0.70), and more modestly to pituitary levels (r=0.60), both being tissues where CCK levels increased in submissive animals. Thus, animals that had the higher CCK levels exhibited more of the affectively negative 20 kHz vocalizations (past research clearly indicates that the submissive animal generate most of these USVs [50]). Since CCK generally tends to facilitate anxiogenic types of responses in past research, this correlation is in a reasonable direction. CCK changes in no other brain region approximated such relationships. In this context it is noteworthy that the 50 kHz positive vocalizations were more modestly, but inversely correlated (−0.53) to those posterior cortical CCK levels. Also, CCK levels in the posterior neocortical region only correlated with levels in one other tissue sample—the pituitary (r=0.47), a structure which, interestingly, was significantly related to both tectum and tegmentum CCK levels (r's=0.41 and 0.44), neither of which was related at all to CCK levels in the posterior (CTX2) cortex (r's=−0.03 and 0.12, respectively). Considering that freezing was also correlated with pituitary CCK levels (r=0.62) and CTX2 (r=0.69), and almost with those of the tegmentum (r=0.53), suggests that there is some large-scale functional relationship between fear behaviors and CCK levels in fairly widely dispersed brain areas.

Perhaps all of these areas participate in the generation of some type of negative affect [10], which could be evaluated with CCK infusions into such areas in conditioned place aversion paradigms. Also, one might consider whether the ability of CCK 4 and 5 fragments in the peripheral circulation to provoke panic attacks in humans [42,46] might be related to activity changes in such regions of the brain. In any event, presently the overall weight of evidence, including the present results, does favor the idea that CCK predominantly mediates some type of negative, anxiety-type, emotional state(s) within the brain [7,18]. Indeed, blockade of CCK2 receptors has proved to be quite effective in reducing short-term anxiety effects, as well as the long-term consequences of trauma and stress in various animal models [1,7].

The "hub" of another CCK system appears to be the caudate-putamen complex and the overlying neo-cortex (CTX 1), since both of these areas are highly correlated with septal, hypothalamic and thalamic CCK levels (among which only septal and hypothalamic levels were positively interrelated with each other in our study). These areas participate in a variety of motivational and attentional functions [37], and this hub may need to be conceptualized differently than the results already discussed. The existence of a seemingly independent relationship of CCK levels comparing the basal forebrain and caudate-putamen to that in the substantia nigra/VTA (r=0.53 and 0.48, respectively) does makes anatomical sense, and may suggest some type of additional independent reward-related functional role [21], especially since this limb of the dopamine/CCK system has previously been related to regulation of sexual behavior [12,33], drug addiction [9,23,53] and other reward processes [11,26]. It would be premature to speculate about such functional relationships without causal manipulations, but it may further highlight the likelihood that several functionally independent CCK brain-behavior control mechanisms may exist within the mammalian brain.

In any event, conceptualization of brain CCK functions with respect to a unitary neuro-psycho-biological concept has seemed remote since the discovery that so many different behavioral and physiological functions were controlled by this prolific neuropeptide system [5,8]. The existence of several distinct receptors (CCK1 and CCK2),

each with quite distinct functions in body and brain [13], and potential additional subtypes within the two major subtypes [10], suggests, once more, that a variety of subfunctions may have evolved from one ancestral neuropeptide system. Although CCK can clearly promote negative emotional behaviors and aversive affective states, especially via CCK2 receptors, the CCK1 part of the system may participate in cognitively arousing, but perhaps emotionally more positive aspects of behavior. Since there is a substantial literature on CCK and learning [10], it is especially intriguing that facilitation of CCK1 transmission improves, while facilitation of CCK2 transmission retards, social memories in rats [30,31].

For the development of new psychiatric therapeutics [39], we may need to better understand the specific brain functions of the various subcomponents of the CCK systems in the brain. For instance, a great deal of excitement still exists about the potential role of CCK in appetite regulation, the first behavioral effect identified for this peptide family [11,43]. However, if one looks at that concept critically in the context of the abundant recent evidence indicating that many negative affective states, from fear to nausea, can reduce feeding, one must question whether the reductions in feeding have been produced by simulating a central state that resembles normal postprandial *satiety*. Indeed, we have previously analyzed such putative satiety effects from a novel alternative motivational perspective—the fact that hunger reduces rough-and-tumble play in juvenile animals while a single meal restores play to normal [48]. If peripheral CCK produces a robust normal feeling of satiety in hungry animals, we would have expected it to also restore play, but it did not [48]. This and the fact that peripherally administered CCK promotes conditioned place aversions [49], highlights the need for more subtle functional experiments in order to fully understand some of the well established behavioral effects of CCK.

Indeed, there is accruing evidence that brain CCK regulates social and emotional processes more than basic motivational ones, such as energy intake. In young animals, blockade of both of the main CCK receptor types facilitates maternal-odor induced place preferences [45], which suggests that pro-social behaviors are facilitated in infant rats by decreasing brain CCK activity. Low CCK activity also facilitates prosocial-emotional regulation [52]. The present data are reasonably consistent with the idea that CCK facilitates anti-social emotional states that may emerge from the stress of aggressive social encounters. CCK appears to be a peptide that reduces the positive emotional impact of social affiliation and attachments [36,52], perhaps in ways opposite to pro-social peptide systems such as endogenous opioids and oxytocin [37].

However, the above studies [36,52] also provide evidence for CCK's role in the establishment and maintenance of positive emotional bonds. How might such seemingly contradictory effects be achieved? One obvious possibility is the oft noted opposite motivational effects of CCK1 and CCK2 receptor systems, with perhaps CCK1 receptors promoting positive social feelings while CCK2 receptors promote distress. Another functional dimension that needs to be considered is that neurochemical states that decrease social reward (e.g., opioid antagonists) can often increase social affiliative behaviors, presumably as instrumental ways to counteract negative affective states [37]. Perhaps similar types of negative affective states provoked by CCK2 activity could promote increased social affiliation that could then be easily, but perhaps mistakenly, taken to reflect an intensified positive social emotional state. Clearly more subtle experiments are needed to disentangle such intriguing issues.

In sum, the ambiguities surrounding the functional role of this system in psychological homeostasis remain abundant. A great deal of additional work is needed before we can refine the concepts already discussed into more definitive ideas about the adaptive functions that CCK subserves within the brain, the body and associated psychiatric/psychosomatic disorders [16,22,44]. However, the present study, one of the first to monitor regional brain CCK changes as a function of a discrete behavioral challenge, indicates that the utilization of this strategy contrasting several additional distinct behaviors may help further clarify the functions of this ubiquitous peptide within the brain.

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