

Electrophysiological Actions of Neuropeptide Y and Its Analogs: New Measures for Anxiolytic Therapy?

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Neuropeptide Y (NPY) has neuromodulatory actions on multiple brain functions including endocrine, behavioral, and circadian processes. Behavioral studies suggest that NPY is a potent anxiolytic; however, little is known about how NPY affects general arousal and/or attention states. The present study evaluated the effects of NPY on spontaneous brain activity as well as auditory processing by using electrophysiological measures. Electroencephalographic (EEG) and event-related potentials (ERPs) were obtained in awake animals after intracerebroventricular administration of NPY (1.0, 3.0 nmol) and two of its analogs, active at Y1 (1.0, 3.0 nmol) and Y2 (1.0, 3.0 nmol) receptor sites. NPY was found to produce dose-related effects on electrophysiological measures. Spectral analyses of the EEG revealed that NPY produced slowing of delta activity (1–2 Hz) in the frontal cortex and high frequency theta activities (6–8 Hz) concomitant with a speeding up of low frequency theta (4–6 Hz) in cortex, hippocampus, and amygdala. At higher doses (3.0 nmols) in addition to shifts in frequency, EEG power was also significantly reduced in all frequencies (0.5–50 Hz) in cortex, and in the higher frequencies (8–32 Hz) in

the amygdala. The Y1 and Y2 agonists had a somewhat different profile of EEG effects than the parent compound. At the 1 nmol dose both agonists were found to produce selective depressions in power in the hippocampus. The 3.0 nmols dose of the Y1 agonist produced decreases in EEG stability, an effect commonly produced by anxiolytic drugs, whereas the Y2 agonist produced increases in EEG stability in cortex and amygdala. Auditory processing, as assessed by ERPs, was affected most significantly in the frontal cortex where dose-dependent decreases in the N1 component of the ERP, a finding also commonly seen after anxiolytics, was found. Y1 and Y2 agonists were also found to significantly reduce the amplitude of the N1 component of the ERP but less so than the parent compound. The electrophysiological and behavioral profiles of NPY and the Y1 agonist resembles those of anxiolytics such as ethanol and benzodiazepines. Taken together these data suggest that electrophysiological measures of the actions of this peptide system may represent a new potentially useful assay for the development of anxiolytic drugs. [Neuropsychopharmacology 17:34–43, 1997] © 1997 American College of Neuropsychopharmacology

KEY WORDS: Amygdala; Dorsal hippocampus; EEG; ERPs; Frontal cortex; Neuropeptides; Spectral analyses

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Neuropeptide Y (NPY) is a hexatriacontapeptide amide, structurally related to pancreatic polypeptide (Tatemoto et al. 1982). It is now well characterized as a neuromodulator in the central nervous system (see Gray and Morley 1986; Mutt et al. 1989; Wahlestedt et al. 1989; Leibowitz 1991; Heilig et al. 1994; Heilig and Widerlöv 1995). The localization of NPY in numerous brain areas supports its role in the regulation of circadian rhythms (Moore and Card 1985; Albers and Ferris 1984), the coordination of endocrine function (Wahles-

tedt et al. 1987; Leibowitz 1988; Abe et al. 1989; Fuxe et al. 1989; Danger et al. 1990; Kalra and Crowley 1992; Catzeflis et al. 1993), modulation of stress and anxiety (Heilig et al. 1989, 1992), and appetitive responses (Clark et al. 1984; Levine and Morley 1984; Stanley and Leibowitz 1984; Stanley et al. 1985; Parrott et al. 1986; Morley et al. 1987). These data support a neuromodulatory role for this neuropeptide in multiple brain functions (Heilig and Widerlöv 1990,1995; Wettstein et al. 1995).

NPY is extensively distributed in brain with particularly dense networks in limbic structures (see de Quidt and Emson 1986a,b; Gustafson et al. 1986; Hendry 1993). In the rat the highest levels of NPY are found in the nucleus accumbens, septum, periaqueductal gray, hypothalamus, whereas moderate amounts are found in the cerebral cortex, hippocampus, amygdala, and caudate-putamen (see Allen et al. 1983; Adrian et al. 1983; Chronwall et al. 1985; de Quidt and Emson 1986 a,b; Shen 1987; Nakagawa et al. 1985). Most NPY neurons in cortex are non-spiny, small interneurons, and in cortical areas NPY has been found to coexist with both somatostatin and GABA (Aoki and Pickel 1989, 1990; Sawchenko et al. 1985; Kowall et al. 1987). In the brainstem, co-localization of NPY with different groups of monoaminergic neurons has been observed (Hendry 1993). There is heterogeneity among NPY receptor subclasses, and in this fast moving field, at least 5 (Y1–Y5) receptors and receptor subtypes have either been suggested or characterized (see Wahlestedt et al. 1986, 1990; Michel 1991; Dumont et al. 1990; Grundemar et al. 1993; Gehlert 1994; Bard et al. 1995; Gerald et al. 1995, 1996; Weinberg et al. 1996).

The behavioral actions of NPY, particularly in rodents, have been extensively described (see Heilig and Widerlöv 1995). In addition to producing increases in feeding behavior, NPY has a marked anxiolytic profile in that when injected into the brain it produces a reduction in “anxiety” in several different animal models (see Heilig et al. 1989, 1992). Most evidence suggests that NPY’s anxiolytic-like action is mediated by Y1 receptors, as the C-terminal fragment of NPY does not produce anxiolytic effects (Heilig et al. 1989) and antisense oligonucleotides targeted to the Y1-receptor message produce marked signs of anxiety when injected into the brain (Wahlestedt et al. 1993). Some progress has also been made in the elucidation of the brain site responsible for NPY’s anxiolytic actions, as local microinjections of NPY into the central nucleus of the amygdala have been found to produce anxiolytic effects without inducing concomitant changes in food intake (Heilig et al. 1993). In addition, antisense inhibition of Y1 receptor expression not only blocks the anxiolytic-like action of NPY, in the amygdala, but also paradoxically increases feeding behaviors (Heilig 1995). Recent studies have postulated that feeding behavior may be modulated by the Y5 receptor (Gerald et al. 1996).

Whereas much progress has been made in describing NPY’s behavioral effects, still little is known concerning

the electrophysiological actions of NPY in the central nervous system in vivo. In one study, cortical EEG was evaluated by visual inspection of polygraph records in five rats (Fuxe et al. 1983). In that study it was found that NPY produced a reduction in desynchronized activity, an increase of synchronized activity and an increase of mixed activity. While this study provides valuable information demonstrating that NPY has an effect on general brain activity, the data are somewhat limited. For instance, it is not known whether NPY produces specific effects on certain frequency bands or whether it produces effects in brain areas other than cortex. In addition, because of the limitations of visual inspection of the EEG it is not certain whether the electrophysiologic effects of NPY resemble the well known profile of anxiolytics. In the present study, we sought to further elucidate the effects of NPY, as well as as specific Y1 and Y2 agonists, on brain electrical activity using quantitative power spectral analysis of EEG recordings in awake, behaving animals. We also investigated NPY’s effects on auditory processing and stimulus discrimination using an event-related potential (ERP) paradigm.

METHODS

Subjects and Surgery

Male Wistar rats (Charles River) weighing from 260–375 g were used as experimental subjects. The rats were housed in pairs with food and water ad lib on a 12-h light/dark cycle (lights on at 6:00 a.m.). At least 3 weeks before the experimental procedures, rats were anesthetized with Nembutal (50 mg/kg + 5 mg intraperitoneally), and recording electrodes were stereotaxically implanted according to the atlas of Pellegrino et al. (1979). One screw electrode was placed in the calvarium overlying the frontal cortex and one overlying the parietal cortex. Unipolar electrodes made of stainless wire (0.25 mm in diameter) were placed in the dorsal hippocampus (DHPC) (AP – 3.0, ML ± 3.0, and DV – 3.0) and the anterior amygdaloid complex (AMYG) (AP – 1.0, ML ± 5.3, DV – 8.5). A grounded reference electrode was placed in the thick bony area of the calvarium 3 mm posterior to lambda, which lies parallel to the cerebellum. One stainless steel cannula (23 gauge) was placed in the lateral ventricle for intracerebroventricular (ICV) injections (AP – 0.6, ML + 2.0, DV – 3.2). The electrode connections were then made to a multipin (Amphe-nol) connector and the entire assembly was anchored to the skull with dental cement.

EEG Recording Procedure

For EEG recordings, the rats are moved from the vivarium in their home cage, and the cage was placed in an electrically shielded, light, sound, and temperature controlled BRS/LVE recording chamber. Rats were adapted

to the chamber before the commencement of the experimental procedures. All studies were carried out between 9:00 a.m. and 2:00 p.m. A connector attached to a microdot cable was used to transfer the monopolar (referred to the lambda ground screw) EEG signals to a polygraph (GRASS). The bandpass for the EEG recordings was set at 1 to 70 Hz with a 60-Hz notch filter in, and the signals were amplified with a 50% gain. For quantification of the EEG, 40 minutes of EEG were digitized (128 Hz) and the power spectra of continuous 4-s epochs determined for a 0.25–64 Hz range. The Fourier-transformed data were then further compressed into eight frequency bands (1–2, 2–4, 4–6, 6–8, 8–16, 16–32, 32–50, 1–50 Hz). Mean power density was calculated in microvolts squared per octave and peak frequency was calculated in Hz. Mean spectral power density over a band was defined as the total power in the band divided by the width of the band. In addition to mean spectral power, the coefficient of variation of mean power (σ/μ), a measure of the “stability” of the EEG was also estimated. Details of the spectral analyses procedures have been previously described (see Ehlers and Havstad 1982).

ERPs were recorded immediately after the 40-min EEG recording. For ERP recordings, the EEG signal was filtered online with the lowpass filter set at 35 Hz and the high-pass filter set at 0.3 Hz. Free field auditory stimuli were presented through a small speaker centered approximately 20 cm above the rat's head. ERPs were elicited with an acoustic “oddball” plus novel paradigm. The tones were generated by a programmable multiple-tone generator, the characteristics of which have been described previously (Polich et al. 1983). The acoustic parameters for this paradigm were two square wave tones (rise/fall times <1 ms): a frequent tone (20 ms, 1 kHz, 70 dB SPL) presented on 84% of the trials and an infrequent tone (20 ms, 2 kHz, 85 dB SPL) presented on 10% of the trials, and a novel noise burst (20 ms, noise, 100 dB SPL) presented on 6% of the trials. The total number of trials in a recording session was 312. Infrequent tones were interspersed with frequent tones, such that no two infrequent tones occurred successively; the noise burst occurred every 16th trial. The digitized epoch for each trial was 1 s and a 0.5–1 s intertrial interval was used in order to reduce habituation.

ERP trials were digitized at a rate of 256 Hz. Trials containing excessive movement artifact were eliminated (<10% of the trials) before averaging. An artifact rejection program was utilized to eliminate individual trials in which the EEG exceeded $\pm 400 \mu\text{V}$. The ERP components were then quantified by a computer-driven program, which identified a peak amplitude (baseline-to-peak) within a standard latency range. The baseline was determined by averaging the 100-ms of prestimulus activity obtained for each trial. The latency of a component was defined as the time of occurrence of the peak amplitude after the onset of the stimulus. The latency windows were initially determined by visual inspection

of the data and then standardized to allow for computer automated peak determinations. Components were labeled by their polarities and latency positions relative to each other. The latency windows for the frontal cortex were: N10, 0–25; P1, 25–75; N1, 50–150; N2, 150–250. The latency windows for the dorsal hippocampus were: N10, 0–25; P1, 25–75; N1, 25–75; P2, 75–150; N2, 150–300; P3A, 150–250; P3B, 250–450. The latency windows for the amygdala were: N10, 0–25; P1, 25–75; N1, 50–100; P2, 75–150; N2, 125–250; P3A, 200–300; P3B, 250–450. These ERP analyses have been demonstrated previously (Ehlers et al. 1991).

Electrophysiological (EEG and ERP) recordings were obtained under seven conditions. In these conditions, rats were treated before the EEG recordings with saline, 1.0, or 3.0 nmols of NPY, 1.0 or 3.0 nmols of the Y1 receptor agonist [Leu³¹, Pro³⁴]-NPY, or 1.0 or 3.0 nmols of the Y2 receptor agonist Des-AA^{7–24} dicyclo(2-27,28-32)[Glu^{2,32}, DAla⁶, DDpr²⁷, Lys²⁸]-NPY all synthesized in the Peptide Biology laboratory, Salk Institute (see Kirby et al. 1993). Peptides were dissolved in sterile saline and were injected using a 30 gauge stainless steel injector connected by a polyethylene tube to a 10- μl Hamilton syringe. A total volume of 5.0 μl was injected in 60 s allowing 1 additional minute for diffusion of the substance before removing the injector. Any rat with resistance to flow into the ventricle, by gravity test, was eliminated from the study. At least 1 week elapsed between peptide injections. The different doses of NPY and the Y1 and Y2 agonists were given in a Latin square design to control for the effects of order. The EEG paper records were scored by hand for the presence of sleep (see Ehlers et al. 1986).

At the end of the experiment, rats were overdosed with nembutal (100 mg/kg) and decapitated. The brains were extracted, frozen, and sectioned (60 μm). The brain slices were then stained with cresyl violet and carefully examined under a light microscope. Data from rats with electrode placement found in unintended brain sites were eliminated from the statistical analyses.

A total of 26 subjects completed the protocol and were used in the statistical analyses. The final number of subjects in each condition ranged from 11 to 20. Not all subjects participated in each condition; each subject received at least saline and two doses of peptide within the latin square design. The effects of the two doses of NPY and the Y1 and Y2 receptor agonists on the EEG, ERPs, and amount of slow wave sleep were compared with the effects of saline injections using one-way within subjects ANOVAs.

RESULTS

EEG Data

No significant difference in amount of time spent in slow wave sleep, as quantified by scoring the EEG

paper records, was found when data from the saline condition were compared to either dose of NPY or its analogs. However, spectral analyses revealed that NPY and its analogs did produce significantly different spectral profiles, as compared to saline, that were brain region specific.

The parent compound, NPY, produced dose-related changes in EEG frequency and power. At the lower dose (1.0 nmol), reductions in EEG power were noted but did not meet significance in any brain region. However, at the higher dose (3.0 nmol) decreases in total power over the entire frequency range (1–50 Hz) ($F = 4.4, df = 1,13, p < .055$), as well as selective significant reductions in the higher theta frequencies (6–8 Hz) ($F = 6.4, df = 1,13, p > .025$) were observed in frontal cortex (see Figure 1). Reductions in power in higher frequencies were also observed in the amygdala (8–16 Hz) ($F = 6.8, df = 1,17, p < .02$; 16–32 Hz) ($F = 6.2, df = 1,17, p < .02$) but not in hippocampus.

Both doses of NPY were also found to provide shifts in peak frequency within a frequency band. At the lower dose, a significant slowing of delta (1–2 Hz) ($F = 22.9, df = 1,13, p < .0001$) frequencies in frontal cortex was present. Slowing of higher theta frequencies (6–8 Hz) was found in frontal cortex, dorsal hippocampus and amygdala (FCTX: $F = 12.5, df = 1,13, p < .004$; DHPC: $F = 6.9, df = 1,17, p < .02$; AMYG: $F = 17.6, df = 1,16, p < .001$). Increases in peak power in the lower theta frequencies were also found in frontal cortex (4–6 Hz) ($F = 7.0, df = 1,13, p < .02$) and amygdala ($F = 11.7, df = 1,16, p < .004$) at the 1.0 nmol dose of NPY. These same shifts in frequency were observed at the 3.0 nmol dose of NPY (1–2 Hz) (FCTX: $F = 17.9, df = 1,13, p < .001$); (4–6 Hz) (FCTX: $F = 5.4, df = 1,13, p < .04$); (6–8 Hz) (FCTX: $F = 5.6, df = 1,13, p < .03$; DHPC: $F = 6.0, df = 1,17, p < .03$; AMYG: $F = 20.3, df = 1,17, p < .0001$). Additional significant shifts toward increases in frequency in the higher ranges (16–32 Hz) (FCTX: $F = 7.5, df = 1,13, p < .02$; DHPC: $F = 7.2, df = 1,17, p < .015$; AMYG: $F = 6.2, df = 1,17, p < .02$); (32–50 Hz) (AMYG: $F = 5.3, df = 1,17, p < .035$) were also noted.

The Y1 and Y2 receptor agonists also produced a profile of frequency and power changes in the EEG. As compared to saline injections, the lower dose (1.0 nmol) of both the Y1 and Y2 receptor agonists caused overall reductions in total spectral power in dorsal hippocampus (1.0–50 Hz) (1 nmol Y1 agonist: $F = 4.6, df = 1,12, p < .05$); (1 nmol Y2 agonist: $F = 7.1, df = 1,9, p < .03$). Selective reductions in power in specific frequency bands in dorsal hippocampus were also found. The Y1 agonist produced decreases in the higher frequencies (6–50 Hz), whereas the Y2 receptor agonist produced decreases in low (1–2, 4–6 Hz) and higher (8–50 Hz) frequencies as seen in Figure 2. The 3-nmol dose of the Y2 agonist did not produce any effects on spectral power in any lead. However, this dose of the Y1 agonist produced increases in power in the low frequencies in all three leads (1–2 Hz) (DHPC: $F = 6.6, df = 1,12, p < .02$; AMYG: $F = 4.6, df = 1,12, p < .05$); (2–4 Hz) (FCTX: $F = 8.8, df = 1,6, p < .03$; DHPC: $F = 5.2, df = 1,12, p < .04$; AMYG: $F = 5.8, df = 1,12, p < .03$); (4–6 Hz) (FCTX: $F = 7.5, df = 1,6, p < .03$; AMYG: $F = 6.3, df = 1,12, p < .03$) as well as the spindle frequencies in cortex (8–16 Hz) ($F = 6.4, df = 1,6, p < .05$).

Similar shifts in peak frequency were found to those observed with the parent compound after administra-

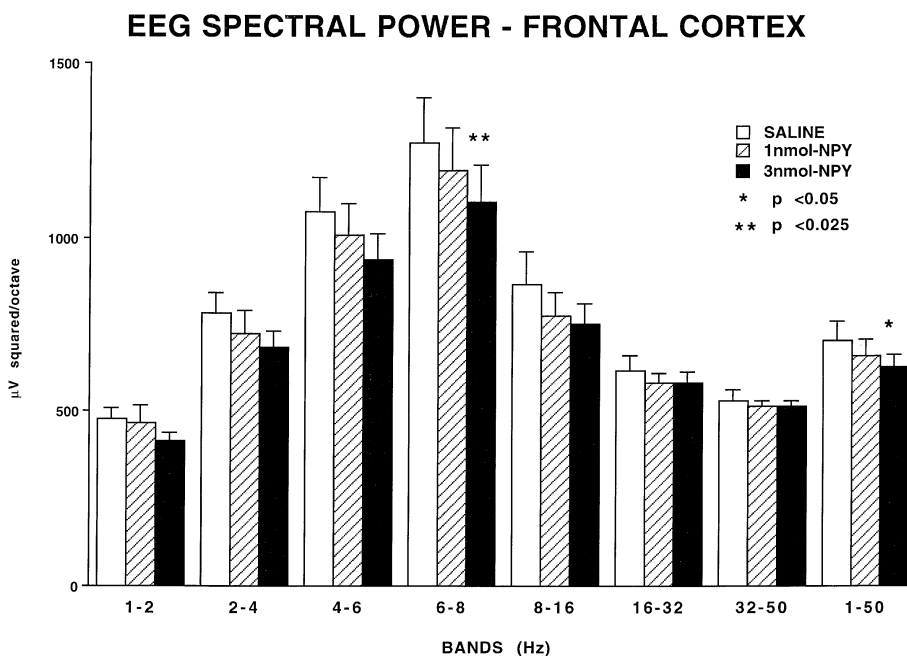


Figure 1. The effects of two doses of NPY (1.0 nmol, hatched bars; 3.0 nmol, solid bars) on EEG spectral power in eight frequency bands in frontal cortex. Significant decreases in overall power (1–50 Hz: $F = 4.4, df = 1,13, p < .05$) and in the higher theta frequencies (6–8 Hz: $F = 6.4, df = 1,13, p < .025$) were noted at the higher doses.

tion of the Y1 agonist. At both doses, decreases in the higher theta frequencies (6–8 Hz) were observed in dorsal hippocampus and amygdala (1 nmol, DHPC: $F = 18.0$, $df = 1,12$, $p < .001$, AMYG: $F = 10.7$, $df = 1,12$, $p < .007$); (3 nmol, DHPC: $F = 6.3$, $df = 1,12$, $p < .03$; AMYG: $F = 12.2$, $df = 1,12$, $p < .004$). Increases in frequency in the higher ranges were also seen after the 1.0 nmol dose of the Y1 agonist (8–16 Hz) (FCTX: $F = 16.5$, $df = 1,7$, $p < .005$; DHPC: $F = 11.6$, $df = 1,12$, $p < .005$). The Y2 agonist did not produce significant shifts in frequency at either dose in any brain area.

The Y1 and Y2 agonists also produced significant changes in the stability of the EEG. EEG stability is defined as the coefficient of variation ($CV_{\sigma/x}$) of power in a particular frequency band. Thus, if there are large fluctuations (with respect to the mean) in power over the recording period, the CV will go up and the EEG is defined as being less stable. The Y1 agonist, like ethanol and benzodiazepines, was found to produce a significant dose-related decrease in EEG stability, whereas the Y2 agonist produced significant dose-related increases as seen in Table 1.

ERP Data

In this study, the presentation of auditory stimuli in the form of infrequent and frequent tones produced a series of waves, including N1 potentials in frontal cortex, and P3 potentials in hippocampus and amygdala, which could be averaged from the EEG and appeared substantially similar to those we have reported previously (see Figure 3).

The administration of NPY had site specific effects on rat ERP components. A significant dose-dependent

reduction in the N1 component, recorded in frontal cortex, to all three tones was a consistent finding as seen in Figure 4. The Y1 and Y2 agonists also produced reductions in the amplitude of the N1 component in frontal cortex but less potently. In the case of the Y1 agonist, N1 amplitude decreases were seen to both doses but only after the noise tone. The Y2 agonist produced reductions in the N1 component, in frontal cortex, but only significantly at the 1-nmol dose to the noise tone and at the 3-nmol dose to the frequent and infrequent tones, as also seen in Figure 4. The parent compound also produced a significant reduction in the P3 component of the ERP, in the amygdala, in response to the noise tone at the 1 nmol dose ($F = 5.5$, $df = 1,17$, $p < .03$), but not significantly at the 3-nmol dose. No significant changes in the late positive component were found after administration of either the Y1 or Y2 agonist.

DISCUSSION

NPY has been shown to have some characteristics similar to those of anxiolytics. For instance, in the present study, NPY was found to induce a slowing of high frequency theta activities (6–8 Hz) concomitant with a speeding up of low frequency theta (4–6 Hz) in cortex, hippocampus and amygdala. We have previously demonstrated, in Wistar rats, that low doses of the benzodiazepine receptor agonist, diazepam (1.5 mg/kg IP), also produces the exact same effects on theta frequency (Robledo et al. 1994). NPY at higher doses (3.0 nmol) was also found to significantly reduce overall spectral power (0.5–50 Hz) in cortex, and in the higher frequencies (8–32 Hz) in the amygdala. Diazepam has also been demonstrated to cause reductions in spectral power in

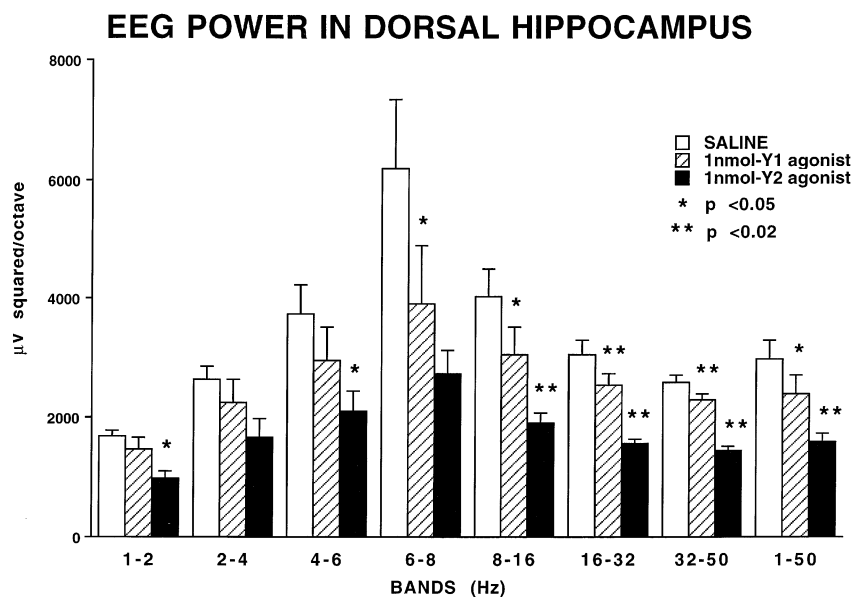


Figure 2. The effects of a 1-nmol dose of the Y1 (*hatched bars*) and Y2 (*solid bars*) agonists on EEG spectral power in eight frequency bands in dorsal hippocampus. Both compounds produced significant reductions in spectral power over the entire frequency range (Y1, 1–50 Hz: $F = 4.57$, $df = 1,12$, $p < .05$) (Y2, 1–50 Hz: $F = 7.11$, $df = 1,9$, $p < .02$).

the slow frequencies in rat (Robledo et al. 1994) and in monkey (Ehlers and Reed 1987), as well as in human subjects during sleep (Borbely 1992).

The Y1 and Y2 agonists had a somewhat different profile of EEG effects than the parent compound. The 3.0-nmol dose of the Y1 agonist produced decreases in EEG stability, whereas the Y2 agonist produced increases in EEG stability in cortex and amygdala. Gehrman and Killam (1978) were the first to suggest that benzodiazepines could be differentiated from other sedative-hypnotics by measures of EEG stability in rhesus monkeys. In subsequent studies in squirrel monkeys (Ehlers and Havstad 1982; Ehlers and Reed 1987), it was confirmed that both diazepam and ethanol can produce a decrease in EEG stability as characterized by an increase in the coefficient of variation of selected frequency bands. Similar EEG data have been described in humans undergoing ethanol challenge (Ehlers et al. 1989). Taken together these data provide additional evidence that NPY has an anxiolytic profile and further that such actions may be preferential for the Y1 receptor.

NPY produces a different profile of EEG changes as compared to other neuropeptides. ICV administration of dynorphin (DYN) has been reported to produce EEG desynchronization concomitant with increases in theta activity, whereas arginine vasopressin (AVP) and neurotensin both produce overall EEG dampening associated with increased "alertness" (Ehlers 1984; Ehlers et

al. 1985; Robledo et al. 1995). Corticotropin-releasing factor (CRF), on the other hand, produced EEG activation followed by the appearance of epileptiform activity (Ehlers et al. 1983). Additionally, growth hormone releasing factor (GRF) has been found to enhance slow waves during sleep (Ehlers et al. 1986). Thus, it appears that NPY has a somewhat unique and specific EEG profile compared to other peptides that have been evaluated.

NPY was also found to produce a significant dose-dependent effect on processing of auditory stimuli as assessed by event related potentials (ERPs). In human subjects, a series of waves of differing polarity and amplitude designated "components" of ERPs are generally obtained from the averaged EEG when a subject is asked to discriminate a target stimulus from a series of background stimuli or are presented frequently and infrequently occurring stimuli of different stimulus characteristics (Roth 1973; Squires et al. 1975; Polich 1987). One such component, which is negative in polarity and occurs around 100 ms after the stimulus, is designated the N1 or N100. In human patients, the N1 has been associated with arousal and attention as well as physical aspects of the stimuli (see Hillyard and Kutas 1983). Another component that is positive in polarity and occurs at approximately 300 ms is called the P3 or P300. The P3 component has been suggested to reflect stimulus evaluation and memory function, (see Donchin and Coles 1988; Verleger 1988). We have demonstrated that

Table 1. EEG Stability (Mean \pm SEM)

Band	Frontal Cortex		Dorsal Hippocampus	
	Saline	3nmol NPY I	Saline	3nmol NPY I
1	0.534 \pm 0.013	0.623 \pm 0.031 ^a	0.410 \pm 0.019	0.480 \pm 0.040 ^a
2	0.454 \pm 0.040	0.662 \pm 0.063 ^c	0.407 \pm 0.028	0.504 \pm 0.039 ^c
3	0.432 \pm 0.023	0.560 \pm 0.046 ^b	0.439 \pm 0.030	0.533 \pm 0.038 ^a
4	0.442 \pm 0.024	0.557 \pm 0.033	0.355 \pm 0.017	0.399 \pm 0.021
5	0.298 \pm 0.012	0.399 \pm 0.053 ^a	0.261 \pm 0.019	0.267 \pm 0.021
6	0.163 \pm 0.007	0.294 \pm 0.086	0.153 \pm 0.011	0.151 \pm 0.010
7	0.184 \pm 0.014	0.299 \pm 0.299	0.134 \pm 0.002	0.135 \pm 0.003
8	0.198 \pm 0.018	0.303 \pm 0.313 ^b	0.184 \pm 0.016	0.226 \pm 0.022 ^b

Band	Frontal Cortex		Amygdala	
	Saline	3nmol NPY II	Saline	3nmol NPY II
1	0.625 \pm 0.049	0.499 \pm 0.030 ^b	0.643 \pm 0.059	0.437 \pm 0.028 ^c
2	0.722 \pm 0.054	0.490 \pm 0.037 ^c	0.559 \pm 0.054	0.384 \pm 0.017 ^c
3	0.772 \pm 0.081	0.495 \pm 0.039 ^b	0.583 \pm 0.069	0.393 \pm 0.020 ^a
4	0.633 \pm 0.041	0.512 \pm 0.056	0.522 \pm 0.060	0.990 \pm 0.042
5	0.527 \pm 0.053	0.432 \pm 0.082	0.368 \pm 0.057	0.223 \pm 0.022 ^a
6	0.264 \pm 0.039	0.282 \pm 0.071	0.157 \pm 0.009	0.163 \pm 0.016
7	0.211 \pm 0.060	0.248 \pm 0.057	0.149 \pm 0.011	0.166 \pm 0.018
8	0.437 \pm 0.039	0.296 \pm 0.047 ^b	0.311 \pm 0.045	0.156 \pm 0.016 ^c

Values are mean \pm SEM.

^a $p < .05$.

^b $p < .02$.

^c $p < .01$.

long latency ERPs can be recorded from monkeys (Ehlers 1988, 1989) and rats (Ehlers et al. 1991; Ehlers and Chaplin 1992; Ehlers et al. 1994) when presented “oddball” tone sequences. In rats, this paradigm has been found to produce early negativities (N1s), as well as late positivities in the 250–400 ms range in parietal cortical areas, dorsal hippocampus, and amygdala that are sensitive to stimuli characteristics (Ehlers and Chaplin 1992) and task (Ehlers et al. 1994).

The most prominent effects of NPY on auditory ERPs were observed in frontal cortex where dose-dependent decreases in the N1 component were observed. Y1 and Y2 agonists were also found to significantly reduce the amplitude of the N1 component of the ERP but less so

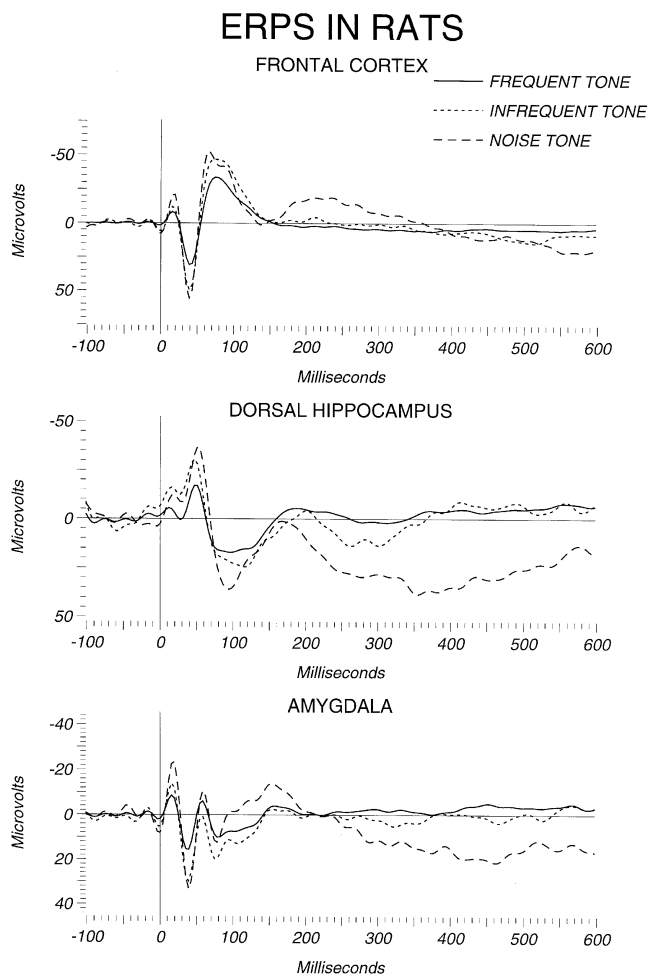


Figure 3. Grand averages of ERPs in 20 rats. ERPs were generated in response to three stimuli: a frequent tone presented on 84% of the trials (solid line), an infrequent tone presented on 10% of the trials (dotted line), and a novel noise burst presented on 6% of the trials (dashed line). Note the prominent negative component arising in frontal cortex with a latency between 75 and 100 ms, designated the N1. In hippocampus and amygdala a late positive component was observed after the frequent and noise tone between 200 and 500 ms designated the P3.

than the parent compound. Reductions in the N1 component of the ERP have previously been reported to occur in monkeys after administration of both diazepam and ethanol (Ehlers 1988; Ehlers et al. 1992). Few studies have evaluated the effects of neuropeptides on ERP components. In one study neurotensin (NT) was found to produce a dose-related increase in the amplitude and

EFFECTS OF NPY ON RAT N1 COMPONENT OF THE ERP

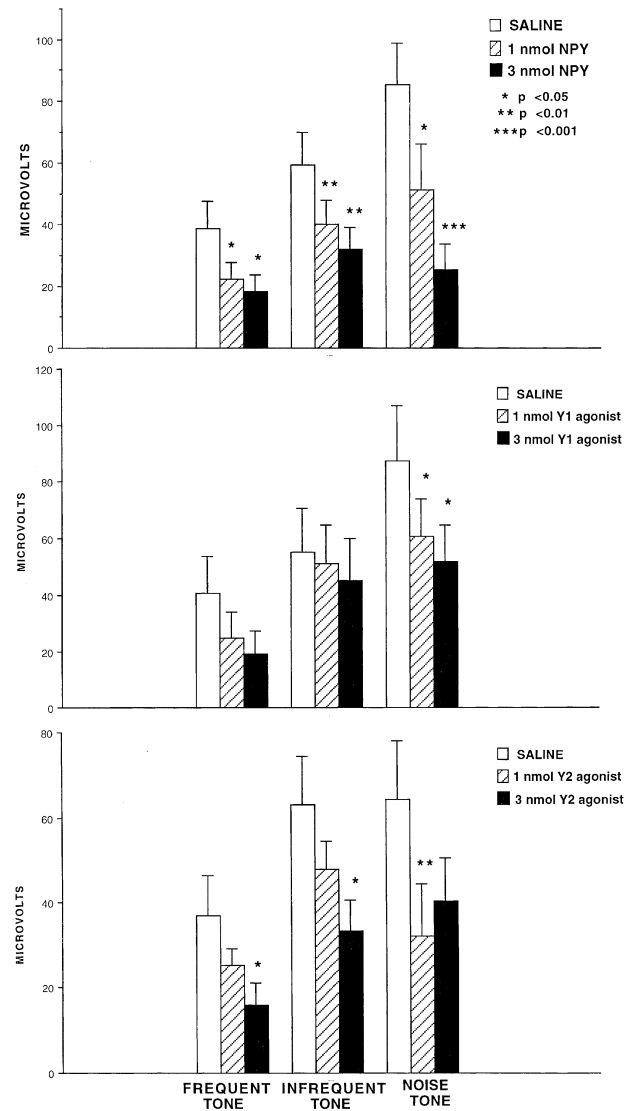


Figure 4. The effects of two doses of: NPY (upper panel), the Y1 agonist (middle panel), and the Y2 agonist (lower panel) on the amplitude of the N1 component of the rat ERP recorded in frontal cortex. In each panel, the first set of bars represents the frequent tone, the second set the infrequent tone, and the third the noise tone. The doses are represented in each panel by hatched bars (1.0 nmol), and solid bars (3.0 nmol). All three compounds were found to produce significant reductions in N1 components (e.g., noise tone: 1 nmol NPY: $F = 6.9, df = 1,15, p < .02$; 1 nmol Y1: $F = 6.0, df = 1,10, p < .03$; Y2: $F = 12.5, df = 1,10, p < .005$).

the area of the rat N1 particularly after the noise burst, a finding exactly opposite to that observed in the present study. These findings suggest that NPY-induced reductions in the N1 component do not reflect nonspecific effects of administration of neuropeptides into the brain. In humans, it has been hypothesized that the auditory N1 component may be an index of attention or the amount of signal information received by the detecting system. For instance, increases in the amplitude of this component have been observed when subjects report a higher confidence in signal detection (Hillyard and Kutas 1983). Therefore, the results observed in this study suggest that NPY may be reducing the salience of auditory stimuli in rats especially to loud, novel stimuli.

Another finding in this study was that NPY, at higher doses, produced a reduction in the late positive potential designated as P3, but only in the amygdala. We have not found that benzodiazepines produce changes in the P3 component of the ERP. However, ethanol has been found to decrease the amplitude of the P3 component at higher doses in cortical areas (Ehlers 1988). In humans, increases in the amplitude of the P3 to passive novel stimuli have been associated with anxiety produced by threat (Grillon and Ameli 1994). It is possible that reductions in P3 in amygdala may reflect the potential for NPY to dampen "threatening" or "emotional" stimuli. Such suggestions would be consistent with behavioral studies demonstrating anxiolytic effects of NPY when administered directly into the central nucleus of the amygdala (Heilig et al. 1993).

In conclusion, the electrophysiological and behavioral profile of NPY and the Y1 agonist resemble those of anxiolytics such as ethanol and benzodiazepines. It has been suggested, based mainly on behavioral and anatomical data, that NPY receptors may prove potential therapeutic drug targets (Wahlestedt and Reis 1993). Taken together, these data provide additional evidence that NPY has an anxiolytic profile and in addition that electrophysiological measures of the actions of this peptide system may eventually provide useful assays for the development of anxiolytic drugs.

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REFERENCES

Abe M, Saito M, Shiumazu T (1989): Neuropeptide Y and norepinephrine injected into the paraventricular nucleus of the hypothalamus activate endocrine pancreas. *Biomed Res* 10:431–436

- Adrian TE, Allen JM, Bloom SR, Ghatei MA, Rossen MN, Roberts GW, Crow TJ, Tatemoto K, Polak, JM (1983): Neuropeptide Y distribution in human brain. *Nature* 306(5943):584–586
- Albers HE, Ferris CF (1984): Neuropeptide Y: Role in light-dark cycle entrainment of hamster circadian rhythms. *Neurosci Lett* 50(1-3):163–168
- Allen YS, Adrian TE, Allen JM, Tatemoto K, Crow TJ, Bloom SR, Polak JM (1983): Neuropeptide Y distribution in the rat brain. *Science* 221(4613):877–879
- Aoki C, Pickel VM (1989): Neuropeptide Y in the cerebral cortex and the caudate-putamen nuclei: Ultrastructural basis for interactions with GAAGAergic and non-GABAergic neurons. *J Neurosci* 9(12):4333–4354
- Aoki C, Pickel VM (1990): Neuropeptide Y in cortex and striatum: Ultrastructural distribution and coexistence with classical neurotransmitters and neuropeptides. *Ann NY Acad Sci* 611:185–205
- Bard JA, Walker MW, Branchek TA, Weinshank RL (1995): Cloning and functional expression of a human Y4 subtype receptor for pancreatic polypeptide, neuropeptide Y and peptide YY. *J Biol Chem* 270(45):26762–26765
- Borbely AA (1992): Effects of hypnotics on sleep regulation and the sleep EEG. *Clin Neuropharmacol* 15(Suppl 1 Pt A):359A
- Catzeflis C, Pierroz DD, Rohner-Jeanrenaud F, Rivier JE, Sizonenko PC, Aubert ML (1993): Neuropeptide Y administered chronically into the lateral ventricle profoundly inhibits both the gonadotropic and the somatotrophic axis in intact adult female rats. *Endocrinology* 132(1):224–234
- Chronwall BM, DiMaggio DA, Massari VJ, Pickel VM, Ruggiero DA, O'Donohue TL (1985): The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience* 15(4):1159–1181
- Clark JT, Kalra PS, Crowley WR, Kalra SP (1984): Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology* 115(1):427–429
- Danger JM, Tonon MC, Jenks BG, Saint-Pierre S, Martel JC, Fasolo A, Breton B, Quirion R, Pelletier G, Vaudry H (1990): Neuropeptide Y: Localization in the central nervous system and neuroendocrine functions. *Fundam Clin Pharmacol* 4:307–340
- de Quidt ME, Emson PC (1986a): Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system. I. Radioimmunoassay and chromatographic characterization. *Neuroscience* 18(3):527–543
- de Quidt ME, Emson PC (1986b): Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system. II. Immunohistochemical analysis. *Neuroscience* 18(3):545–618
- Donchin E, Coles MGH (1988): Is the P300 component a manifestation of context updating? *Behav Brain Sci* 11:357–374
- Dumont Y, Fournier A, St-Pierre S, Schwartz TW, Quirion R (1990): Differential distribution of neuropeptide Y1 and Y2 receptors in the rat brain. *Eur J Pharmacol* 191(3):501–503
- Ehlers CL (1984): Role of selected neuropeptides in the development of epileptiform discharges. In Fariello RG,

- Morselli PL, Lloyd KG, Quesney LF, Engel J Jr (eds), Neurotransmitters, Seizures, and Epilepsy II. New York, Raven Press, pp 295–304
- Ehlers CL (1988): ERP responses to ethanol and diazepam administration in squirrel monkeys. *Alcohol* 5:315–320
- Ehlers CL (1989): EEG and ERP responses to naloxone and ethanol in monkeys. *Prog Neuropsychopharmacol Biol Psychiatry* 13:217–228
- Ehlers CL, Havstad JW (1982): Characterization of drug effects on the EEG power spectral band time series analysis. *Psychopharmacol Bull* 18:43–47
- Ehlers CL, Henriksen SJ, Wang M, Rivier J, Vale W, Bloom FE (1983): Corticotropin releasing factor produces increases in brain excitability and convulsive seizures in rats. *Brain Res* 278:332–336
- Ehlers CL, Kaneko WM, Robledo P, Lopez A (1994): Long latency event-related potentials in rats: Effects of task and stimulus parameters. *Neuroscience* 62(3):759–769
- Ehlers CL, Reed TK (1987): Ethanol effects on EEG spectra in monkeys: Comparison to morphine and diazepam. *Electroencephalogr Clin Neurophysiol* 66:317–321
- Ehlers CL, Reed TK, Wang M, Lebrun CJ, Koob GF (1985): EEG effects of subcutaneous and intracerebroventricular injections of arginine vasopressin in the rat. *Psychopharmacology* 87:430–433
- Ehlers CL, Reed TK, Henriksen SJ (1986): Effects of corticotropin-releasing factor and growth hormone-releasing factor on sleep and activity in rats. *Neuroendocrinology* 42:467–474
- Ehlers CL, Wall TL, Chaplin RI (1991): Long latency event related potentials in rats: Effects of dopaminergic and serotonergic depletions. *Pharmacol Biochem Behav* 38:789–793
- Ehlers CL, Chaplin RI (1992): Long Latency event-related potentials in rats: The effects of changes in stimulus parameters and neurochemical lesions. *J Neural Transm* 88:61–75
- Ehlers C, Kaneko WM, Wall TL, Chaplin RI (1992): Effects of dizocilpine (MK-801) and ethanol on the EEG and event-related potentials (ERPs) in rats. *Neuropharm* 31(4):369–378
- Ehlers CL, Wall TL, Schuckit MA (1989): EEG spectral characteristics following ethanol administration in young men. *EEG Clin Neurophysiol* 73:179–187
- Fuxe K, Agnati LF, Härfstrand A, Zini I, Tatemoto K, Pich EM, Hökfelt T, Mutt V, Terenius L (1983): Central administration of neuropeptide Y induces hypotension bradypnea and EEG synchronization in the rat. *Acta Physiol Scand* 118:189–192
- Fuxe K, Agnati LF, Härfstrand A, Eneroth P, Cintra A, Tinner B, Pich EM, Aronsson M, Bunnemann B, Lang R, Ganten D (1989): Studies on the neurochemical mechanisms underlying the neuroendocrine actions of neuropeptide Y. In Mutt V, Hökfelt T, Fuxe K, Lundberg JM (eds), *Neuropeptide Y*. New York, Raven Press, pp 115–136
- Gehlert DR (1994): Subtypes of receptors for neuropeptide Y: Implications for the targeting of therapeutics. *Life Sci* 55:551–562
- Gehrmann JE, Killam JF Jr (1978): Studies of central functional equivalence—Time varying distributions of power in discrete frequency bands of the EEG as a function of drug exposure. *Neuropharmacology* 17:747–759
- Gerald C, Walker MW, Vaysse PJ, He C, Branchek TA, Weinshank RL (1995): Expression cloning and pharmacological characterization of a human hippocampal neuropeptide Y/peptide YY Y2 receptor subtype. *J Biol Chem* 270(45):26758–26761
- Gerald C, Walker MW, Criscione L, Gustafson EL, Batzl-Hartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA, Weinshank RL (1996): A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature* 382 (6587):168–171
- Gray TS, Morley JE (1986): Neuropeptide Y: Anatomical distribution and possible function in mammalian nervous system. *Life Sci* 38:389–401
- Grillon C, Ameli R (1994): P300 assessment of anxiety effects of processing novel stimuli. *Int J Psychophysiol* 17(3):205–217
- Grundemar L, Sheikh SP, Wahlestedt C (1993): Characterization of receptor types for neuropeptide Y and related peptides. In Colmers WF, Wahlestedt C (eds), *The Biology of Neuropeptide Y and Related Peptides*. Totowa, NJ, Humana Press, p 197
- Gustafson EL, Card JP, Moore RY (1986): Neuropeptide Y localization in the rat amygdaloid complex. *J Comp Neurol* 251(3):349–362
- Heilig M (1995): Antisense inhibition of neuropeptide Y (NPY)-Y1 receptor expression blocks the anxiolytic-like action of NPY in amygdala and paradoxically increases feeding. *Regul Pept* 59(2):201–205
- Heilig M, Koob GF, Ekman R, Britton KT (1994): Corticotropin-releasing factor and neuropeptide Y: Role in emotional integration *TINS* 17(2):80–85
- Heilig M, McLeod S, Brot M, Heinricks SC, Menzaghi F, Koob GF, Britton KT (1993): Anxiolytic-like action of neuropeptide Y: Mediation by Y1 receptors in amygdala, and dissociation from food intake effects. *Neuropsychopharmacology* 8(4):357–363
- Heilig M, McLeod S, Koob GF, Britton KT (1992): Anxiolytic-like effect of neuropeptide Y (NPY), but not other peptides in an operant conflict test. *Regul Pept* 41(1):61–69
- Heilig M, Söderpalm B, Engel JA, Widerlöv E (1989): Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology* 98(4):524–529
- Heilig M, Widerlöv E (1990): Neuropeptide Y: An overview of central distribution, functional aspect, and possible involvement in neuropsychiatric illnesses. *Acta Psychiatr Scand* 82:95–114
- Heilig M, Widerlöv E (1995): Neurobiology and clinical aspects of Neuropeptide Y. In Nemeroff CB, De Souza EB (eds), *Critical Reviews in Neurobiology*. 9(2-3):115–136
- Hendry JHC (1993): Organization of neuropeptide Y neurons in the mammalian central nervous system. In Colmers WF, Wahlestedt C (eds), *The Biology of Neuropeptide Y and Related Peptides*. Totowa, NJ, Humana Press, p 65
- Hillyard SA, Kutas M (1983): Electrophysiology of cognitive processing. *Annu Rev Psychol* 34:33–61

- Kalra SP, Crowley WR (1992): Neuropeptide Y: A novel neuroendocrine peptide in the control of pituitary hormone secretion, and its relation to lutenizing hormone. *Front Neuroendocrinol* 13:1–46
- Kirby DA, Koerber SC, Craig AG, Feinstein RD, Delmas L, Brown MR, Rivier JE (1993): Defining structural requirements for neuropeptide Y receptors using truncated and conformationally restricted analogues. *J Med Chem* 36:385–393
- Kowall NW, Ferrante RJ, Beal MF et al. (1987): Neuropeptide Y, somatostatin, and reduced nicotinamide adenine dinucleotide phosphate diaphorase in the human striatum: A combined immunocytochemical and enzyme histochemical study. *Neuroscience* 20(3):817–828
- Levine AS, Morley JE (1984): Neuropeptide Y: A potent inducer of consummatory behavior in rats. *Peptides* 5(6):1025–1029
- Leibowitz SF (1988): Hypothalamic paraventricular nucleus: Interaction between alpha 2-noradrenergic system and circulating hormones and nutrients in relation to energy balance. *Neurosci Biobehav Rev* 12:101–109
- Leibowitz SF (1991): A brain neuropeptide Y: An integrator of endocrine, metabolic and behavioral processes. *Brain Res Bull* 27:333–337
- Michel MC (1991): Receptors for neuropeptide Y: Multiple subtypes and multiple second messengers. *Trends Pharmacol Sci* 12(10):389–394
- Moore RY, Card JP (1985): Visual pathways and the entrainment of circadian rhythms. *Ann NY Acad Sci* 453:123–133
- Morley JE, Hernandez EN, Flood JF (1987): Neuropeptide Y increases food intake in mice. *Am J Physiol* 253(3 Pt. 2):R516–522
- Mutt V, Hökfelt T, Fuxe K, Lundberg JM (eds) (1989): Neuropeptide Y. *Karlnska Institute Nobel Conference Series*. New York, Raven Press
- Nakagawa Y, Shiosaka S, Emson PC, Tohyama M (1985): Distribution of neuropeptide Y in the forebrain and diencephalon: An immunohistochemical analysis. *Brain Res* 361(1-2):52–60
- Parrott RF, Heavens RP, Baldwin BA (1986): Stimulation of feeding in the satiated pig by intracerebroventricular injection of neuropeptide Y. *Physiol Behav* 36(3):523–525
- Pellegrino LJ, Pellegrino AS, Cushman AJ (1979): *A Stereotaxic Atlas of the Rat Brain*. New York, Plenum Press
- Polich J, Fisher A, Starr A (1983): A programmable multi-tone generator. *Behav Res Methods Instrum* 15:39–41
- Polich J (1987): Comparison of P300 from a passive tone sequence paradigm and an active discrimination task. *Psychophysiology* 24:41–46
- Robledo P, Kaneko WM, Ehlers CL (1995): Effects of neurotensin on EEG and event-related potentials in the rat. *Psychopharmacology* 118:410–418
- Robledo P, Lumeng L, Li T-K, Ehlers CL (1984): Effects of MK 801 and diazepam on the EEG of P and NP rats. *Alcohol Clin Exp Res* 18(2):363–368
- Roth WT (1973): Auditory evoked responses to unpredictable stimuli. *Psychophysiology* 10:125–137
- Sawchenko PE, Swanson LW, Grzanna R, Howe PR, Bloom SR, Polak JM (1985): Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. *J Comp Neurol* 241(2):138–153
- Shen CL (1987): Distribution of neuropeptide Y immunoreactivity in the forebrain of the rat. *Proc Natl Sci Council Repub China Part B* 11(2):115–127
- Squires NK, Squires KC, Hillyard SA (1975): Two varieties long-latency positive waves evoked by unpredictable auditory stimuli in man. *Electroencephalogr Clin Neurophysiol* 38:387–401
- Stanley BG, Daniel DR, Chin AS, Leibowitz SF (1985): Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate ingestion. *Peptides* 6(6):521–524
- Stanley BG, Leibowitz SF (1984): Neuropeptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci* 34(26):2635–2642
- Tatemoto K, Carlquist M, Mutt V (1982): Neuropeptide Y a novel brain peptide with structured similarities to peptide YY and pancreatic polypeptide. *Nature* 296(5858):659–660
- Verleger R (1988): Event-related potentials and cognition: A critique of the context updating hypothesis and an alternative interpretation of P3. *Behav Brain Sci* 11:343–356
- Wahlestedt C, Ekman R, Widerlöv E (1989): Neuropeptide Y (NPY) in the central nervous system: Distribution, effects and possible relationship to neurological and psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 13(1-2):31–54
- Wahlestedt C, Grundemar L, Kakanson R, Heilig M, Shen GH, Zikowsak-Grojec Z, Reis DJ (1990): Neuropeptide Y receptor subtypes, Y1 and Y2. *Ann NY Acad Sci* 611:7–26
- Wahlestedt C, Pich EM, Koob GF, Yee F, Heilig M (1993): Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. *Science* 259(5094):528–531
- Wahlestedt C, Reis DJ (1993): Neuropeptide Y-related peptides and their receptors—are the receptors potential therapeutic drug targets. *Annu Rev Pharmacol Toxicol* 33:309–352
- Wahlestedt C, Skagerberg G, Edman R, Heilig M, Sundler F, Hakanson R (1987): Neuropeptide Y (NPY) in the area of the paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. *Brain Res* 417:33–38
- Wahlestedt C, Yanaihara N, Hakanson R (1986): Evidence for different pre- and postjunctional receptors for neuropeptide Y and related peptides. *Regul Pept* 13(3-4):307–318
- Weinberg DH, Sirinathsinghji DJ, Tan CP, Shiao LL, Morin N, Rigby MR, Heavens RH, Rapoport DR, Bayne ML, Cascieri MA, Strader CD, Linemeyer DL, MacNeil DJ (1996): Cloning and expression of a novel neuropeptide Y receptor. *J Biol Chem* 271(28):16435–16438
- Wettstein JG, Earley B, Junien JL (1995): Central nervous system pharmacology of neuropeptide Y. *Pharmacol Ther* 65(3):397–414