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Electrophysiological Response to Neuropeptide Y (NPY): In Alcohol-Naive Preferring and Non-preferring Rats

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EHLERS, C. L., C. SOMES, L. LUMENG AND T. K. LI. *Electrophysiological response to neuropeptide Y (NPY): In alcohol-naive preferring and non-preferring*. PHARMACOL BIOCHEM BEHAV **63**(2) 291–299, 1999.—Electroencephalograms (EEGs) and event-related potentials (ERPs) to auditory stimuli were recorded following intracerebroventricular administration of neuropeptide Y (saline, NPY: 1.0, 3.0 nmol) in two lines of rats that have been genetically selected for alcohol preferring (P) or non-preferring (NP) behaviors. Previous studies have demonstrated that NPY has a distinct electrophysiological profile that is similar to that of ethanol. In outbred Wistar rats, both NPY and ethanol produced highly significant decreases in the amplitude and increases in the latency of the N1 component of the ERP to all three auditory stimuli. Because the N1 has been associated with attention, these data suggest that both NPY and alcohol may diminish attentional processes. In the present study, NPY-induced decreases in N1 amplitude were also found, but only to the frequently presented tone. This suggests that both P and NP rats may have attenuated responses to NPY's effects on attention/arousal. Like outbred Wistars, P and NP rats were also found to have significant NPY-induced increases in N1 latency in the cortex and hippocampus. However, in the amygdala, while P rats evidenced increases in N1 latency and decreases in N1 amplitudes, NP rats displayed the opposite effects. Spectral analysis revealed that NPY also produced differential EEG responses in P and NP rats. In previous studies in outbred Wistar rats NPY has been found to produce slowing of delta (1–2 Hz) frequencies at the 1-nmol dose and reductions in power, particularly in the higher frequencies in the amygdala, at the 3-nmol dose. This electrophysiological profile is not unlike what is seen following alcohol and benzodiazepines and is associated with anxiolysis. P rats were found to have this general pattern of EEG responses to NPY but attenuated suggesting that they may have reduced responses to electrophysiological measures of the anxiolytic effects of NPY. In contrast, NP rats had NPY-induced EEG effects in amygdala and frontal cortex that were opposite to those seen in P rats. These opposing responses to NPY tended to produce a "normalization" of the power differences that existed between the two rat lines at baseline. Taken together with previous findings that P rats have decreased NPY concentrations in limbic and frontal cortical sites, these data suggest that differences in the regulation of NPY neurons may contribute to the expression of behavioral preference for ethanol consumption in these rat lines. © 1999 Elsevier Science Inc.

Alcohol-preferring rats Electroencephalogram (EEG) Event-related potentials (ERPs) spectral analysis Alcoholism Neuropeptide Y (NPY)

NEUROPEPTIDE Y (NPY), a hexatriacontapeptide amide structurally related to pancreatic polypeptide (67), has now been well characterized as a neuromodulator in the brain (25,27,32,37,52,68). NPY's behavioral actions, particularly in rodents, have been extensively described (31,32). NPY stimulates ingestive behaviors (7,38,47,54–56,64,65) and, when injected into the central nervous system, has been shown to produce a reduction in "anxiety" in rodents (4,28,29). The anxiolytic actions of NPY are most likely mediated by Y1 receptors (28), as administration of antisense oligonucleotides targeted to the Y1-receptor message produce marked signs of anxiety behaviors (69) and diminish the anxiety reducing actions of NPY in the amygdala (26). Additionally, local microinjections of NPY into the central nucleus of the amygdala

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have also been found to produce anxiolytic effects, also providing evidence that this nucleus may be one locus of the effects of NPY's anxiolytic actions (30).

Alcohol has also been demonstrated to reduce behavioral signs of anxiety in rodents (3,8,36,59). Although the mechanisms underlying alcohol's anxiolytic effects are are not entirely known, interactions with both CRF (1,46,61) and GABA receptors (42,49,50,60) have been suggested. A possible interaction of alcohol with NPY receptors has also recently been suggested based on similarities in their electrophysiological and behavioral profiles (19,21).

The selectively bred alcohol preferring (P) and non-preferring (NP) rats provide a model to further explore genetically influenced responses to alcohol, and to search for the underlying neuronal mechanisms that may mediate those responses. The present study was undertaken to explore electrophysiological responses to NPY between P and NP rats. In these experiments, EEG and ERP responses to centrally administered saline and NPY (1 and 3 nmol) were evaluated in P and NP rats to determine whether they differed in response to this neuropeptide.

METHOD

Subjects

The experimental subjects were 37 male rats, 20 P and 17 NP bred at Indiana University. Rats had not been formally preference tested to avoid previous exposure to ethanol. However, since about the 20th generation, the differences in drinking scores between P and NP rats have been quite stable. For example, the drinking scores (g/kg/day: mean ± SD) at S27 were: P males 6.2 ± 1.5 and NP males 0.9 ± 0.7 (41). In the S31 generation, the scores are: P males 5.7 ± 0.16 and NP males 0.5 ± 0.08. There are within line variations, which are largely environmental in origin. P and NP rats from Indiana University were received at The Scripps Research Institute weighing 281–410 g.

At least 2 weeks prior to the experimental procedures, the rats were surgically prepared with recording electrodes and intracerebroventricular (ICV) cannulae. Rats were anesthetized (Nembutal, 50 mg/kg IP) and stainless steel single wire electrodes were aimed at the dorsal hippocampus (AP −3.0, ML ±3.0, DV −3.0) and amygdala (AP 1.0, ML = 5.3, DV −8.5). A 23-gauge stainless steel guide cannula was also aimed at the lateral ventricle (P 1.0, L 1.5, V 4.6). Screw electrodes were placed in the calvarium overlying the frontal cortices. A "reference" electrode, which was grounded, was also placed in the thick bony area of the calvarium 3 mm posterior to lambda, which lies parallel to the cerebellum. In all animals, electrode connections were made to a multipin (Amphenol) connector and the entire assembly was anchored to the skull with dental acrylic.

Peptide Administration

Eleven P rats and 16 NP rats survived the surgical procedures. There was no weight difference between the rats that survived and those that did not survive surgery. The average weights of the P and NP rats also did not differ significantly. NPY was synthesized in the Peptide Biology laboratory, Salk Institute (35). NPY was dissolved in sterile saline and 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 for each infusion, allowing 1 additional min for diffusion of the substance before removing the injector. Any rats with resistance to flow into the ventricle, by gravity test, were eliminated from the study. Rats were only tested once for each condition in a randomized dose design. All studies were run between 1000 and 1400 h. Following the study, rats were decapitated and the brains removed and quickly frozen. The brains were subsequently assayed for peptide contents as previously described (20).

EEG Recordings and Analysis

For EEG recordings, the rats were placed with their cagemates in a electrically shielded light, sound, and temperature controlled BRS/LVE recording chamber. All animals were adapted to being in the chamber prior to peptide/saline administration. On the test days following saline or peptide administration, rats were singly placed in the chamber and a connector attached to a microdot cable was used to transfer the monopolar (referred to the lambda ground screw) EEG signals to a polygraph (Sensorium). The signals were amplified and bandpassed (0.5–70 Hz) and the EEG as well as the calibration signals were transferred to a Macintosh computer. Forty minutes of EEG were collected and 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 seven frequency bands (1–2, 2–4, 4–6, 6–8, 8–16, 16–32, and 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. Mean power density and peak frequency was calculated for each band, for each of the two experimental conditions as described previously (13).

ERP Recordings and Analysis

ERPs elicited by auditory stimuli were presented through a small speaker centered approximately 20 cm above the rat's head at approximately 60 min following peptide administration. EEG signals were recorded on a polygraph (Sensorium), amplified using low pass of 70 Hz and and a time constant of 0.3 s, and then transferred to a Macintosh computer. ERPs were elicited by an acoustic "oddball" plus noise paradigm. The tones utilized were generated by a programmable multiple-tone generator, the characteristics of which have been described previously (58). The acoustic parameters were three tones [rise/fall times <1 ms): a frequently presented tone (20 ms, 1 kHz, 70 dB sound pressure level (SPL)] presented on 84% of the trials, and a rare tone (20 ms, 2 kHz, 85 dB SPL) presented on 10% of the trials and a noise tone presented on 6% of the trials (20 ms, noise, 100 dB SPL). Rare tones were interspersed with standards such that no two rare tones occurred successively. The noise tone occurred every 16th trial. The digitizing epoch for each trial was 1 s, and a variable 0.5–1-s intertrial interval was used to reduce habituation. There was a total of 312 trials in a recording session.

ERP recordings were analyzed for the three conditions (saline, NPY 1, and NPY3). The ERP trials were digitized at a rate of 256 Hz. Trials containing excessive movement artifact were eliminated prior to averaging (<5% of the trials). An artifact rejection program was utilized to eliminate individual trials in which the EEG exceeded ±250 μV. The ERP components were quantified by computer by identifying 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 am-

plitude within a latency window. 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 solely by their polarities and latency positions relative to each other. The components focused on were those shown previously to be sensitive to the effects of NPY, namely the N1 and P3 components. The latency windows for those components in cortex were: N1, 50–150 ms; and P3, 300–450 ms. The windows for dorsal hippocampus were: N1, 25–75 ms; and P3, 300–450 ms. The latency windows for amygdala were: N1 50–150 ms; and P3, 300–425 ms. These ERP analyses have been described previously (11,16,17).

For statistical analysis of the data, mean power and peak frequency of the seven EEG frequency bands and amplitude and latencies for the N1 and P3 ERP components were compared between subjects (preference: P vs. NP) for the three drug conditions (drug: saline vs. NPY 1, 3) using a two-factor analysis of variance (ANOVA) to determine if P and NP rats differed in their electrophysiological responses to NPY. When significant results were found in the two-factor analyses a univariate post hoc ANOVA was performed.

RESULTS

EEG Responses to NPY in P and NP Rats

A comparison of EEG power values in the seven frequency bands revealed that differences existed between P and NP rats in response to NPY (pref × drug). These significant differences were brain area specific. Differences were found in frontal cortex and the amygdala, but not in hippocampus or parietal cortex. As seen in Fig. 1, P and NP rats differed in their responses in the delta (1–2 Hz) frequencies in frontal cortex where Ps were found to have lower overall delta frequencies [pref: $F(1, 18) = 17.24$, $p < 0.001$] and reductions following NPY [pref × drug: $F(2, 36) = 3.88$, $p < 0.03$) compared to NP rats whose delta frequencies increased following NPY administration. Differences were also seen in the theta frequencies (6–8 Hz) in frontal cortex where P rats experienced drops in theta frequency, whereas NPs had no change or increases [pref × drug: $F(2, 36) = 4.75$, $p < 0.015$]. A reduction in power in the higher (beta) frequencies (16–32 Hz) in frontal cortex was also observed following NPY in P rats but not in NPs [pref × drug: $F(2, 36) = 7.18$, $p < 0.002$].

As illustrated in Fig. 2, P and NP also differed in their EEG responses to NPY in amygdala. Overall reductions in power in response to NPY were found in the amygdala in P rats, whereas NPs had NPY-induced increases in power in the theta (6–8 Hz) [pref × drug: $F(2, 36) = 3.29$, $p < 0.05$], and higher (8–16) [pref × drug: $F(2, 36) = 3.37$, $p < 0.045$], (16–32 Hz) [pref × drug: $F(2, 36) = 5.21$, $p < 0.01$] frequency bands as well as in total power (1–32 Hz) [pref × drug: $F(2, 36) = 3.43$, $p < 0.04$].

ERP Responses to NPY in P and NP Rats

The presentation of auditory stimuli in the form of infrequent, and frequent "pure" tones as well as louder "noise" tones produced a series of waves including N1 potentials in frontal cortex as well as N1 and P3 potentials in hippocampus, amygdala, and parietal cortex. Differences in the amplitude and latency of the N1 and P3 components to administration of NPY in P and NP rats were found. In previous studies in outbred Wistar rats, significant decreases in the amplitude of the N1 component in cortical areas was observed to all three tones (19). In P and NP rats, however, this decrease was limited to the frequently presented tone [drug, FCTX: $F(2, 42) = 3.5$, $p < 0.04$, PCTX: $F(2, 44) = 3.261$, $p < 0.05$]. Post hoc analyses revealed that these effects were significant for both doses of NPY in frontal cortex [FCTX, NPY 1: $F(1, 22) = 5.4$, $p < 0.03$; NPY 3: $F(1, 22) = 5.44$, $p < 0.03$] and parietal cortex [PCTX, NPY 1: $F(1, 23) = 5.23$, $p < 0.03$; NPY3: $F(1, 23) = 4.1$, $p < 0.05$].

Increases in the latency of the N1 component were found following NPY to all three tones in frontal cortex [drug:FCTX, frequent tone: $F(2, 42) = 12.3$, $p < 0.0001$; infrequent tone: $F(2, 42) = 3.8$, $p < 0.03$; noise tone: $F(2, 42) = 3.23$, $p < 0.05$]. Post hoc analyses revealed that these effects were significant for the NPY 1 dose following the frequent tone, $F(1, 22) = 24.5$, $p < 0.00006$ and infrequent tone, $F(1, 22) = 6.7$, $p < 0.017$, and for both doses following the noise tone [NPY1: $F(1, 22) = 4.2$, $p < 0.05$; NPY3: $F(1, 22) = 4.9$, $p < 0.04$]. Increases in the N1 latency were also found for the DHPC to the frequent tone [drug: $F(2, 40) = 8.1$, $p < 0.001$], that was significant for the NPY1 dose [post hoc: $F(1, 21) = 5.6$, $p < 0.03$]. In the AMYG, increases in N1 latency were also seen to the infrequent tone (drug: $F(2, 42) = 3.48$, $p < 0.04$] at the NPY1 dose [post hoc: $F(1, 22) = 7.1$, $p < 0.014$].

P and NP rats, however, did differ in their N1 latency and amplitude responses to NPY in the amygdala. NP rats displayed decreases in N1 latency (noise tone, AMYG pref × drug: $F(2, 42) = 9.66$, $p < 0.0001$] and increases in N1 amplitude [frequent tone, AMYG pref × drug: $F(2, 42) = 4.27$, $p < 0.02$], whereas Ps displayed increases in N1 latency and decreases in N1 amplitude.

The latency of the P3 component was also found to be differentially affected by NPY in P and NP rats. As seen in Fig. 3, P rats evidenced increases in the latency of the P3 component in hippocampus, whereas NPs displayed decreases [pref × drug, infrequent tone: $F(2, 42) = 9.27$, $p < 0.0001$; noise tone pref × drug: $F(2, 42) = 4.8$, $p < 0.014$]. Similar findings were observed for the P3 latency in amygdala [noise tone, pref × drug: $F(2, 42) = 9.68$, $p < 0.0001$] and parietal cortex [infrequent tone, pref × drug: $F(2, 42) = 5.67$, $p < 0.006$].

DISCUSSION

Animal models provide the means by which genetic factors that may influence differences in alcohol drinking behavior might be explored (10,22,24). Genetic selection experiments at Indiana University have produced two lines of rats which significantly differ in their ethanol consumption (40,43). Ethanol-preferring (P) rats have been shown, in a free-choice situation, to voluntarily consume 6–10 g/kg of ethanol day, whereas non-preferring (NP) rats generally consume less than 1 g/kg day (39). In addition to consuming large quantities of alcohol under free choice, P rats will also work to obtain ethanol through operant responding in the presence of food and water (51,57). P rats will also self-infuse ethanol intragastrically (72), and intracranially (23) have been shown to develop tolerance and dependence (70).

One brain peptide system that may mediate preference for ethanol is Neuropeptide Y (NPY). There are several studies that provide evidence to suggest a relationship between alcohol and NPY neural systems. NPY stimulates ingestive behaviors producing enhancement of both food and sucrose intake (45). Both food deprivation and food restriction have been found to increase NPY concentrations (5,62) and NPY mRNA (2,53) in the paraventricular and arcuate nuclei of the hypothalamus. Additionally, it has been shown that 4 weeks

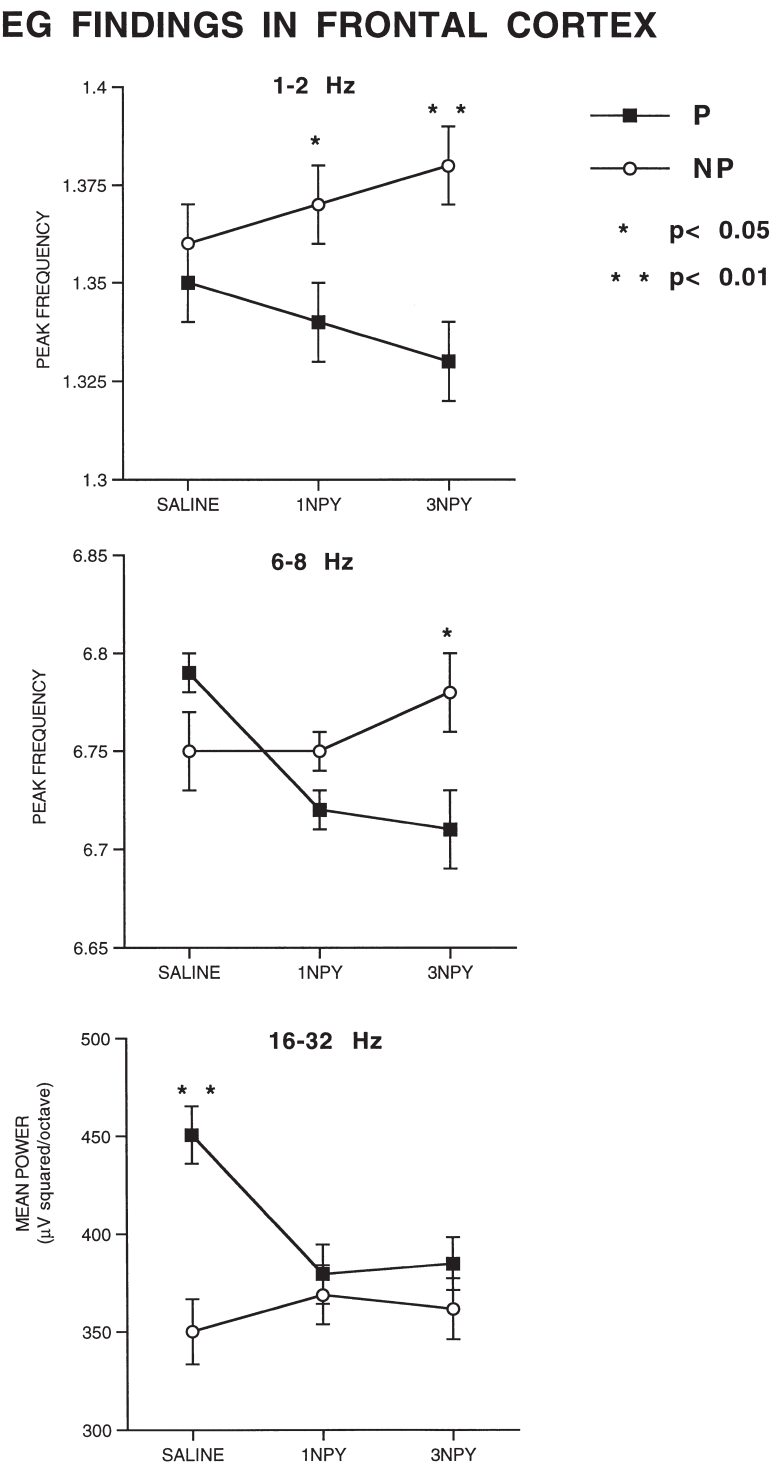

FIG. 1. EEG response to NPY (1 and 3 nmol, saline) in P (closed squares) and NP rats (open circles) in frontal cortex. As seen in the upper graph, Ps were found to have lower overall delta frequencies [pref: $F(1, 18) = 17.24$, $p < 0.001$] as well as reductions following NPY [pref $\times$ drug: $F(2, 36) = 3.88$, $p < 0.03$] compared to NP rats whose delta frequencies increased following NPY administration [post hoc ANOVA: P vs. NP 1NPY, $F(1, 21) = 6.7$, $p < 0.017$] [post hoc ANOVA: 3NPY: $F(1, 19) = 19.0$, $p < 0.0003$]. The middle graph displays EEG responses in the theta frequencies in frontal cortex where P rats experienced drops in theta frequency, whereas NPs had no change or increases [pref $\times$ drug: $F(2, 36) = 4.75$, $p < 0.015$] [post hoc ANOVA: P vs. NP 3NPY, $F(1, 19) = 6.9$, $p < 0.017$]. In the bottom graph reductions in power in the higher (beta) frequencies (16–32 Hz) in frontal cortex following NPY in P rats but not in NPs (pref $\times$ drug: $F(2, 36) = 7.18$, $p < 0.002$] [post hoc ANOVA: P vs. NP SAL, $F(1, 21) = 7.97$, $p < 0.01$] are illustrated.

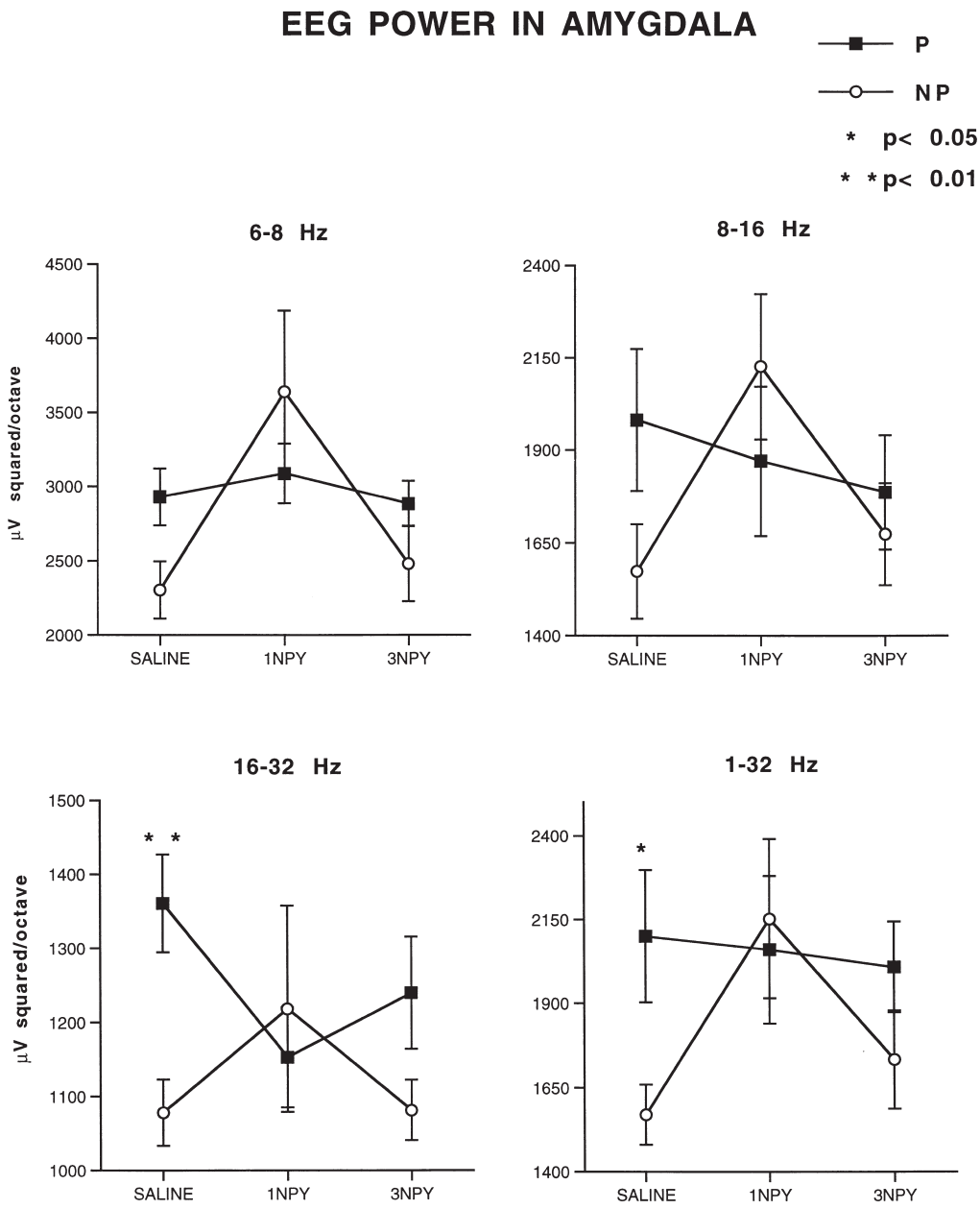

FIG. 2. EEG response to NPY (1 and 3 nmol, saline) in P (closed squares) and NP rats (open circles) in amygdala. Overall reductions in power in response to NPY were found in the amygdala in P rats, whereas NPs had NPY-induced increases in power in the theta (upper left graph) (6–8 Hz) [pref × drug: $F(2, 36) = 3.29$, $p < 0.05$], and higher (upper right graph) (8–16) [pref × drug: $F(2, 36) = 3.37$, $p < 0.045$], (lower left graph) (16–32 Hz) [pref × drug: $F(2, 36) = 5.21$, $p < 0.01$] (post hoc ANOVA: P vs. NP SAL, $F(1, 22) = 10.5$, $p < 0.004$] frequency bands as well as in total power (lower right graph) (1–32 Hz) [pref × drug: $F(2, 36) = 3.43$, $p < 0.04$] [post hoc ANOVA: P vs. NP SAL, $F(1, 22) = 4.63$, $p < 0.04$].

of withdrawal from chronic ethanol exposure can cause significantly higher NPY concentrations in hypothalamus compared to control animals (20). One potential interpretation of these findings is that exposure to high levels of alcohol followed by withdrawal produces changes in NPY similar to a food deprivation/restriction paradigm. These data also suggest the possibility that hypothalamic levels of NPY could potentially modulate alcohol intake.

Additional studies have shown that brain levels of NPY differ between the P and NP lines. Lowered levels of NPY have been found in limbic areas and frontal cortex (20), whereas higher levels of NPY are seen in hypothalamic sites (34). There is some evidence that P rats may be more "anxious" than NP rats (66). Therefore, the lowered levels of NPY seen in frontal cortex and limbic sites may provide part of the substrate for the increased anxiety seen in P rats compared to

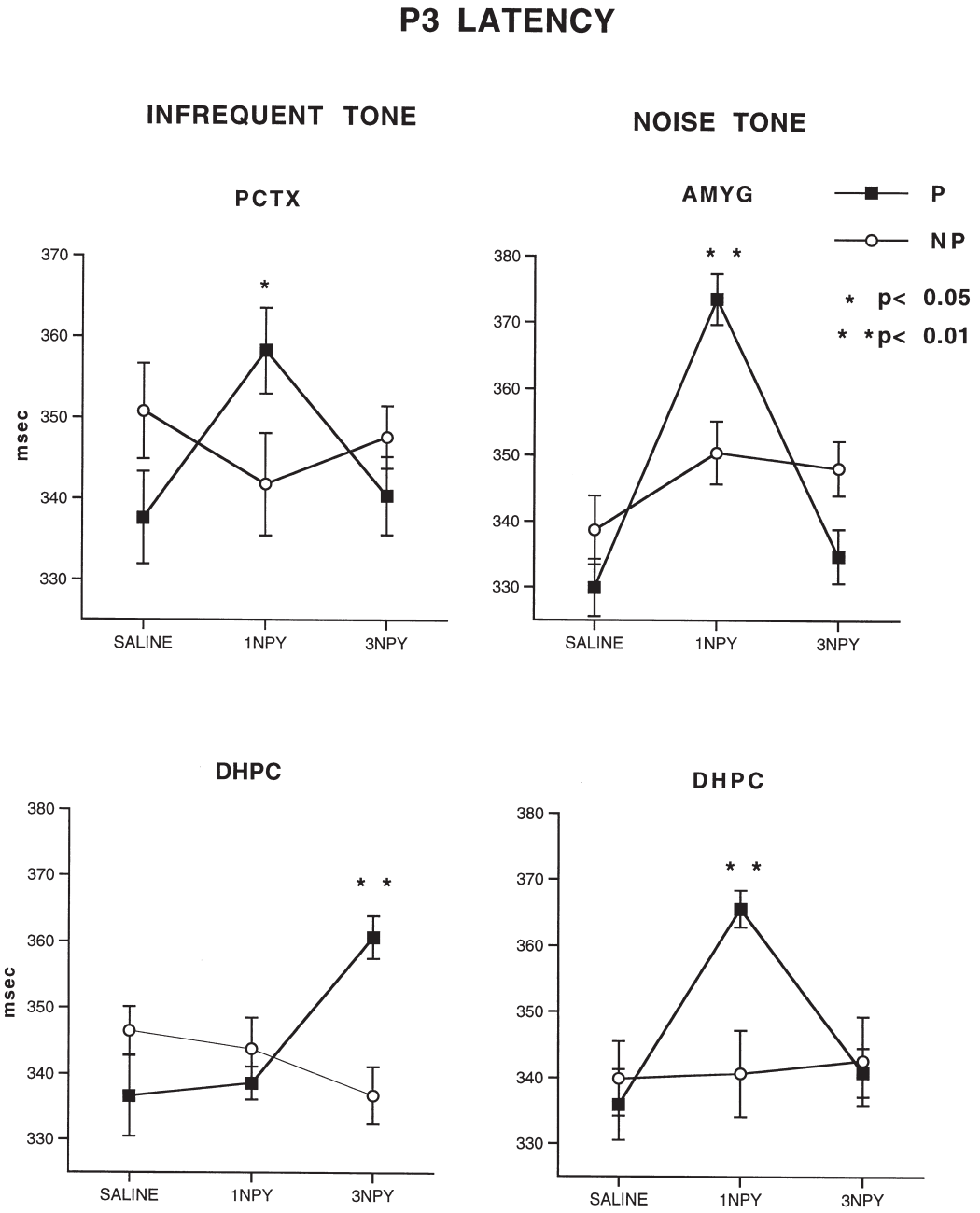

FIG. 3. ERP response to NPY (1 and 3 nmol, saline) in P (closed squares) and NP rats (open circles) in parietal cortex (Pctx) (left upper graph), amygdala (AMYG) (right upper graph), dorsal hippocampus (DHPC) (left and right lower graphs) in response to the infrequently presented "pure" tone (left) and the "noise" tone (right). P rats evidenced increases in the latency of the P3 component in hippocampus, particularly at the higher dose, whereas NPs displayed decreases [pref × drug, infrequent tone: $F(2, 42) = 9.27$, $p < 0.0001$, post hoc P vs. NP NPY3 $F(1, 22) = 22.6$, $p < 0.0001$, noise tone pref × drug: $F(2, 42) = 4.8$, $p < 0.014$, post hoc P vs. NP NPY1 $F(1, 21) = 10.7$, $p < 0.004$]. Similar findings were observed for the P3 latency in amygdala [noise tone, pref × drug: $F(2, 42) = 9.68$, $p < 0.0001$, post hoc P vs. NP NPY1: $F(1, 21) = 14.7$, $p < 0.001$], and parietal cortex [infrequent tone, pref × drug: $F(2, 42) = 5.67$, $p < 0.006$, post hoc P vs. NP NPY1 $F(1, 22) = 4.1$, $p < 0.05$].

NP rats. Increased hypothalamic NPY could additionally explain the increased alcohol intake seen in the P line. Further evidence is provided by studies demonstrating a quantitative trait locus (QTL) with a lod score of 8.6 accounted for 11% of the total phenotypic variability, and approximately one-third of the genetic variability between the P and NP lines has been mapped to a region of chromosome 4 where the gene for NPY is located (6).

Electrophysiological responses to neuropeptide Y (NPY) were investigated in P and NP rats in the present study, be-

cause previous studies had demonstrated that a distinct electrophysiological profile for NPY (19) that is remarkably similar to that of ethanol (21). NPY, like ethanol, produces decreases in the amplitude and increases in the latency of the N1 component of the ERP. In human subjects, the N1 has been associated with arousal and attention as well as physical aspects of the stimuli (9,33). In the present study, NPY-induced decreases in N1 amplitude were also found, but only to the frequent tone. In previous studies in outbred Wistar rats, highly significant NPY-induced decreases in N1 amplitude were observed to all three tones. This suggests that P and NP rats may have attenuated responses to NPY's effects on attention/arousal. NP rats were also found to have some NPY-induced EEG and ERP effects in amygdala and frontal cortex that were opposite to those seen in P rats. These opposing responses to NPY tended to produce a "normalization" of differences that existed between the two rat lines at baseline. Taken together, these data suggest that both P and NP rats differ from outbred Wistars in their physiological responses to NPY with P rats having a pattern similar to outbred Wistars but attenuated, and NP rats displaying an opposing pattern. It is not known if P rats have differential behavioral responses to NPY, however, these electrophysiological data suggest that this would be a useful area of exploration. In addition, the exact relationship between preference for alcohol and brain concentrations of NPY or physiological response to NPY are not known. Thus, it is not clear whether the disparate responses to NPY in the P and NP lines contribute to a preference for or a nonpreference for ethanol.

In previous studies evaluating EEG responses to central peptide administration, P rats were observed to have a significantly enhanced EEG response to corticotropin releasing factor (CRF) as opposed to NP rats (12). One interpretation of these results is that P rats may have a heightened response to "stress" peptides and an attenuated response to "anxiolytic" peptides. The relationship between stress, central CRF/NPY responses, and alcohol preference behaviors in P rats may be coupled to their differences in response to ethanol. P rats compared to NP rats have been shown to have a less sedating or more arousing response to ethanol by several electrophysiological (18,48) and behavioral (44,71) studies. These studies in rats are also consistent with studies in humans, where it has been shown that men at high risk for alcoholism display less intense behavioral (63) and electrophysiological (14,15) responses to ethanol. In theory, under conditions of stress, central release of CRF may produce more intense "stress" responses in subjects at genetic risk for alcoholism, requiring these subjects to drink larger doses of ethanol to obtain the same "tension reducing" effects. This combination of enhanced response to CRF and attenuated response to NPY/ethanol in P rats may theoretically form the substrate for the "genetic component" of the tension reduction hypothesis of alcohol use and abuse.

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