Evidence for *in vivo* Interactions between Neuropeptide Y–related Peptides and σ Receptors in the Mouse Hippocampal Formation

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Recently, it was proposed that neuropeptide Y (NPY) and peptide YY (PYY) could act as endogenous ligands for σ binding sites, as both NPY and PYY competed with high affinity (nanomolar) for ^3H -(+)-SKF 10,047 binding sites in rat brain membrane homogenates (Roman et al., 1989). However, various laboratories failed to replicate these *in vitro* findings. In order to clarify this apparent discrepancy and investigate further possible interactions between NPY and σ related sites, we evaluated the effects of NPY, PYY and homologs, as well as nonrelated peptides, on *in vivo* ^3H -(+)-SKF 10,047 binding parameters in the mouse hippocampal formation. As expected, haloperidol (2 mg/kg), a prototypical σ receptor ligand, competed for 90% of *in vivo* hippocampal labeling observed following a peripheral intravenous injection of ^3H -(+)-SKF 10,047. Intracerebroventricular injections of 300–3000 pmol of either NPY, PYY, NPY_{2–36}, or the Y₁ agonist Leu³¹,Pro³⁴-NPY inhibited significant proportions (17–35%) of haloperidol-sensitive *in vivo* ^3H -(+)-SKF 10,047 hippocampal labeling. However, a Y₂ receptor agonist, NPY_{13–36}, and nonrelated peptides such as neurotensin and vasoactive intestinal polypeptide, as well as adrenalin, failed to alter *in vivo* ^3H -(+)-SKF 10,047 hippocampal binding. It thus appears that NPY, PYY, and a selective Y₁ agonist can interact in a concentration-dependant manner, with *in vivo* ^3H -(+)-SKF 10,047 labeling in the mouse hippocampal formation. This effect demonstrates selectivity as a Y₂ agonist, unrelated peptides, and adrenalin failed to alter *in vivo* σ labeling. This *in vivo* interaction may be relevant to some of the respective biological actions of NPY and σ -related molecules.

[Key words: neuropeptide Y, peptide YY, sigma receptors, N-allylnormetazocine (NANM or SKF 10,047), hippocampus, *in vivo* binding]

The existence of sigma (σ) receptor binding sites in the brain has generated much interest over the past few years likely because of their possible involvement in psychosis-related behaviors (for recent reviews, see Deutsch et al., 1988; Junien and

Leonard, 1989; Snyder and Largent, 1989; Chavkin, 1990; Musacchio, 1990; Walker et al., 1990; Ferris et al., 1991; Itzhak and Stein, 1991a; Su, 1991). This assumption is mostly based on the fact that high densities of σ binding sites are found in various limbic structures, including the hippocampus (Gundlach et al., 1986; Contreras et al., 1987b; McLean and Weber, 1988), and to the high affinity of some neuroleptics (e.g., haloperidol and remoxipride, but not sulpiride) for σ sites (Su, 1982, 1991; Tam and Cook, 1984; Taylor and Dekleva, 1987). This suggested the possible development of a new class of σ -related antipsychotic drugs devoid of dopaminergic activity (Su, 1986; Taylor and Dekleva, 1987; Snyder and Largent, 1989). Additional support for this hypothesis came from observations suggesting that certain σ drugs such as *N*-allylnormetazocine (NANM or SKF 10,047) induced hallucinations in man (Keats and Telford, 1964) and delirium in the chronic spinal dog (Martin et al., 1976; Vaupel, 1983), although the genuine σ nature of these effects remains to be fully established (Quirion et al., 1987; Walker et al., 1990).

The abundance and discrete distribution of σ sites in the mammalian brain may also reveal the possible existence of endogenous σ -related ligands. Early on, few groups (Quirion et al., 1984; Su et al., 1986; Contreras et al., 1987a; Su and Vaupel, 1988; Zhang et al., 1988; Connor and Chavkin, 1991, 1992) reported on the possible isolation of such molecules, although their purification to homogeneity still remains to be accomplished. Thus, the report by Roman et al. (1989) suggesting that peptides of the pancreatic polypeptide family, namely, neuropeptide Y (NPY) and polypeptide YY (PYY), were able to compete with high affinities for *in vitro* ^3H -(+)-SKF 10,047/ σ binding sites generated tremendous interests. However, it has been difficult to replicate these findings using well-established *in vitro* membrane binding assays (Quirion et al., 1991; Tam and Mitchell, 1991).

On the other hand, evidence has recently accumulated supporting the existence of interactions between NPY and σ -related systems, *in vivo*. For example, Monnet et al. (1990a,b, 1992a–c) have shown that both NPY and σ agonists, in a haloperidol-sensitive manner, are able to potentiate *N*-methyl-D-aspartate (NMDA)-induced firing in the pyramidal CA3 subfield of the hippocampal formation (but see McQuiston and Colmers, 1991; Connick et al., 1992). Additionally, NPY and a selective σ ligand, JO 1784, can decrease intestinal transport apparently via haloperidol-sensitive σ receptors (Rivière et al., 1990). Moreover, it appears that NPY and homologs, as well as various σ ligands, are able to potentiate NMDA-induced ^3H -noradrenaline release in rat hippocampal slices (Monnet et al., 1991; Ro-

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man et al., 1991). Accordingly, we decided to investigate further possible interactions between NPY-related peptides and σ receptor sites in the mouse hippocampus using an *in vivo* binding approach; the hippocampal formation was chosen as this region is most enriched with both σ - (Gundlach et al., 1986; Contreras et al., 1987b; McLean and Weber, 1988; Weissman et al., 1988, 1990) and NPY (Martel et al., 1986, 1990a; Lynch et al., 1989; Dumont et al., 1990, 1992, 1993) receptors. Our results suggest that NPY, PYY, and a selective agonist of the Y_1 receptor subtype can interact with a certain proportion of *in vivo* ^3H -(+)-SKF 10,047 labeling in the mouse hippocampus.

Materials and Methods

Materials. ^3H -(+)-SKF 10,047 (53 Ci/mmol; Comission Energie Atomique, Saclay, France) was provided by the Institut de Recherches Jouveinal, Fresnes, France. All NPY-related peptides as well as neurotensin and vasoactive intestinal polypeptide (VIP) were synthesized in our laboratories as described earlier (St.-Pierre et al., 1981; Martel et al., 1990b). Adrenalin, haloperidol, trizma base (Tris), NaCl, and polyethylenimine (PEI) were purchased from Sigma Chemicals (St. Louis, MI). MK-801 was from Research Biochemicals Inc. (Natick, MA), ether and GF/B Whatman filters were obtained from Fisher (Montréal, Canada), and Ecolite TM+ was purchased from ICN Biochemicals (Montréal, Canada).

Animals. Male CD-1 mice (20–24 gm) were purchased from Charles River (St.-Constant, Québec, Canada). Animals were housed in a temperature- and humidity-controlled room on a 12 hr:12 hr light/dark cycle, and were fed ad libitum with standard laboratory chow and tap water. They were kept under these conditions for at least 24 hr prior to the experiment. Animal care was according to protocols and guidelines approved by McGill University and the Canadian Council for Animal Care.

Binding experiments. For *in vivo* binding experiments, mice were injected at time (t) = 0 with 2 mg/kg haloperidol (100 μl , i.p.), MK-801 (0.05, 0.1, 0.2, or 0.5 mg/kg 100 μl , i.p.), or saline (0.9% NaCl, 100 μl , i.p.) in order to determine the proportion of *in vivo* ^3H -(+)-SKF 10,047 labeling that can be related to σ sites. At t = 15 min, under light ether anesthesia, animals received either a peptide injection (3 μl , i.c.v.) or saline (3 μl , i.c.v.). Fifteen minutes later (t = 30 min), animals were injected in the tail vein with 5 μCi of ^3H -(+)-SKF 10,047 (200 μl , i.v.). Animals were killed by dislocation at t = 60 min. Brains were then rapidly removed and hippocampi were dissected on ice and immediately homogenized in 1 ml of ice-cold buffer (Tris-HCl 5 mM, pH 7.4 at 4°C) using a Teflon/glass Potter-Elvehjem probes (12 strokes). Immediately thereafter, 1 ml of ice-cold Tris-HCl buffer was added to this homogenate before filtration of 1.5 ml of this preparation through three GF/B glass filters (pretreated for at least 24 hr in buffer containing 0.05% PEI) under vacuum using a 12-hole Millipore CAT apparatus. Filters were then washed twice with 5 ml of ice-cold incubation buffer and then placed in vials containing 5 ml of Ecolite TM+ scintillation cocktail. Radioactivity was determined using a Beckman LS1800 counter with 40% efficiency. The remaining 0.5 ml of the homogenate was kept for protein determination (Lowry et al., 1951). Statistical analyses were performed using the Student's t test, p < 0.05 being considered statistically significant.

Results

In preliminary experiments we failed to obtain evidence for *in vitro* interactions between NPY, PYY, and ^3H -(+)-SKF 10,047/ σ sites as the two peptides, in concentrations ranging from 10^{-11} to 10^{-6} M, did not compete for *in vitro* ^3H -(+)-SKF 10,047 binding in mouse brain membrane preparations. These findings confirmed data obtained earlier in the rat brain (Quirion et al., 1991; Tam and Mitchell, 1991).

In vivo ^3H -(+)-SKF 10,047 binding in the mouse hippocampus

An equivalent of approximately 15 fmol/mg of protein was detected in the mouse hippocampus following an injection in the tail vein of 5 μCi of the σ ligand ^3H -(+)-SKF 10,047. In the

Figure 1. ^3H -(+)-SKF 10,047-related radioactive material present in the mouse hippocampal formation following a peripheral intravenous injection (5 μCi per 200 μl , tail vein; total n = 51). ^3H -(+)-SKF 10,047 binding is most likely related to σ sites as haloperidol (2 mg/kg, i.p.; n = 11) almost completely inhibited (90%) labeling in the mouse hippocampus. Similarly, although to a lesser extent, intracerebroventricular injections of NPY (1500 pmol; n = 23) significantly inhibited hippocampal ^3H -(+)-SKF 10,047 labeling. *, p < 0.005; **, p < 0.001.

presence of haloperidol (2 mg/kg, i.p.) residual ^3H -(+)-SKF 10,047 hippocampal binding accounted for only 1.48 fmol/mg of protein, revealing that approximately 90% (13.52 fmol/mg protein) of total labeling was sensitive to the prototypical σ ligand (Fig. 1). The NMDA/phencyclidine (PCP) receptor ligand MK-801 did not inhibit the specific ^3H -(+)-SKF 10,047 labeling in the mouse hippocampal formation at intraperitoneal doses of 0.05, 0.1, 0.2, and 0.5 mg/kg (n = 26; data not shown).

Competition by NPY-related peptides

As shown in Figure 3, NPY, NPY₂₋₃₆, Leu³¹,Pro³⁴-NPY (a purported Y_1 agonist; Fuhlendorff et al., 1990), and PYY were able to displace approximately one-third of specific *in vivo* ^3H -(+)-SKF 10,047 labeling in the mouse hippocampus. The fragment NPY₁₃₋₃₆ (a Y_2 agonist; Wahlestedt et al., 1986) and the analog desamido-NPY (NPY-OH) were inactive. As shown in Figure 2, various doses of NPY (300, 600, 1000, 1500, and 3000 pmol, i.c.v.) demonstrated that this peptide inhibited the *in vivo* hippocampal labeling of ^3H -(+)-SKF 10,047 in a dose-related manner. The minimal effective dose appears to be in the range of 600 pmol while a maximal effect was observed at 1500 pmol, a higher dose of NPY (3000 pmol) failing to produce a greater effect (Fig. 2).

The respective potency of various NPY-related peptides to inhibit *in vivo* ^3H -(+)-SKF 10,047 labeling in the mouse hippocampus is shown in Figure 3. At doses of 1500 and 3000 pmol, the homolog PYY most potently inhibited specific ^3H -(+)-SKF 10,047 labeling, its effects being apparently even greater than that of NPY. In contrast, NPY-OH failed to inhibit ^3H -(+)-SKF 10,047 labeling (Fig. 3A), as expected for this biologically inactive analog (Wahlestedt et al., 1986).

The fragment NPY₂₋₃₆ was able to mimic the effect of the full NPY molecule on ^3H -(+)-SKF 10,047 labeling (Fig. 3B). However, a shorter C-terminal fragment, NPY₁₃₋₃₆, a purported Y_2 agonist (Wahlestedt et al., 1986), failed to alter ^3H -(+)-SKF 10,047 labeling at 1500 pmol (Fig. 3B). On the other hand, the Y_1 agonist Leu³¹,Pro³⁴-NPY, was the most potent competitor of *in vivo* ^3H -(+)-SKF 10,047 labeling tested in this study, with a profile similar to that of NPY, namely, a dose of 1500 pmol inducing a greater effect than that of 3000 pmol (Figs. 2, 3B).

Figure 2. Dose dependency demonstration of the effect of NPY on *in vivo* ^3H -(+)-SKF 10,047 labeling of the mouse hippocampal formation. Intracerebroventricular injections of increasing doses of NPY significantly inhibited *in vivo* ^3H -(+)-SKF 10,047 labeling (total $n = 51$) with a minimally effective dose in the range of 600 pmol ($n = 23$); a lower dose (300 pmol; $n = 25$) inhibited binding to a nonsignificant extent; 1000 pmol ($n = 23$) produced a greater inhibition than that of 600 pmol, while maximal effectiveness was observed at 1500 pmol ($n = 23$), with 3000 pmol ($n = 11$) being somewhat less effective. *, $p < 0.05$; **, $p < 0.005$.

Specificity of NPY for *in vivo* hippocampal ^3H -(+)-SKF 10,047 labeling

As a means to assess the specificity of the effect of NPY and related analogs for *in vivo* ^3H -(+)-SKF 10,047 labeling, we investigated next the effects of two other classes of peptides, namely, neurotensin (1500 pmol) and VIP (1500 pmol), and of the well-known vasoconstrictor/neurotransmitter adrenalin (1500 and 3000 pmol). These three molecules failed to alter *in vivo* ^3H -(+)-SKF 10,047 labeling in the mouse hippocampus at doses tested here (Fig. 4).

Discussion

The present study demonstrates that (1) NPY and its homolog PYY dose-dependently inhibited the *in vivo* binding of the prototypical σ ligand ^3H -(+)-SKF 10,047 in the mouse hippocampal formation; (2) a similar effect is also seen with a Y_1 (Leu 31 ,Pro 34 -NPY) but not a Y_2 (NPY $_{13-36}$) receptor agonist, suggesting receptor subtype specificity; (3) a biologically inactive NPY analog, NPY-OH, failed to alter *in vivo* ^3H -(+)-SKF 10,047 labeling; and (4) other biologically active polypeptides such as neurotensin and VIP, which are known to induce various cerebrovascular and neuronal effects (Rioux et al., 1981; Wilson et al., 1981), and adrenalin failed to modulate *in vivo* ^3H -(+)-SKF 10,047 labeling, demonstrating the specificity of the interactions between certain NPY-related peptides and *in vivo* ^3H -(+)-SKF 10,047 binding in the mouse hippocampus.

The hippocampal labeling observed following an intravenous injection of ^3H -(+)-SKF 10,047 is very likely representing binding to σ sites that are most abundant in this region (Largent et al., 1986; Contreras et al., 1987b; Weissman et al., 1990). While (+)-SKF 10,047 can bind with relatively high affinities to both PCP and σ sites (Su, 1982; Largent et al., 1986; Quirion et al., 1987; McCann et al., 1989), only the σ component is sensitive to haloperidol (Su, 1982; Tam and Cook, 1984). Moreover, four different doses (0.05, 0.1, 0.2, and 0.5 mg/kg, i.p.; $n = 26$) of the high-affinity NMDA/PCP receptor ligand MK-801 (Loo et al., 1987) did not reduce the specific *in vivo* ^3H -(+)-SKF 10,047 labeling in the mouse hippocampus. Thus, the high sensitivity

Figure 3. *A*, Comparative potencies of NPY, PYY, and NPY-OH to inhibit *in vivo* ^3H -(+)-SKF 10,047 labeling (total $n = 51$) in the mouse hippocampal formation. PYY at 1500 pmol ($n = 13$) and 3000 pmol ($n = 8$) was slightly more potent than NPY at 1500 pmol ($n = 23$) to inhibit labeling, while NPY-OH at 1500 pmol ($n = 7$) and 3000 pmol ($n = 13$) failed to produce any significant effect. *, $p < 0.005$. *B*, Comparative potencies of NPY $_{1-36}$, C-terminal fragments (NPY $_{2-36}$ and NPY $_{13-36}$), and the analog Leu 31 ,Pro 34 -NPY (LP) to inhibit *in vivo* ^3H -(+)-SKF 10,047 labeling (total $n = 51$) in the mouse hippocampal formation. NPY $_{2-36}$ at 1500 pmol ($n = 20$) and 3000 pmol ($n = 10$) significantly inhibited ^3H -(+)-SKF 10,047 binding albeit to a lower extent than the full peptide, at 1500 pmol ($n = 23$). A prototypical Y_2 fragment, NPY $_{13-36}$ (1500 pmol, $n = 14$), failed to exert any effect on ^3H -(+)-SKF 10,047 labeling, while the Y_1 agonist Leu 31 ,Pro 34 -NPY potently competed for labeling at 1500 pmol ($n = 14$) and 3000 pmol ($n = 7$). *, $p < 0.05$; **, $p < 0.005$.

of *in vivo* hippocampal ^3H -(+)-SKF 10,047 labeling to haloperidol, together with the ineffectiveness of MK-801 to inhibit the specific ^3H -(+)-SKF 10,047 labeling in the mouse hippocampus *in vivo*, confirms the σ nature of the related sites. Our results also extend previously reported *in vivo* ^3H -(+)-SKF 10,047/ σ -related binding data (Ferris et al., 1988; Tam et al., 1988; Weissman et al., 1990). However, it is unclear if, under the present assay conditions, *in vivo* ^3H -(+)-SKF 10,047 labeling represents binding to more than one subtype of σ site. Recently, evidence has been obtained to support the existence of at least two classes (σ_1 and σ_2) of σ sites (Hellewell and Bowen, 1990; Itzhak and Stein, 1991a,b; Itzhak et al., 1991; Su et al., 1991; Quirion et

al., 1992; Vilner and Bowen, 1992). These two putative subtypes demonstrate similar affinity for haloperidol, while (+)SKF 10,047 apparently preferentially labels the $\sigma 1$ sites (Hellewell and Bowen, 1990; Itzhak and Stein, 1991b; Itzhak et al., 1991; Su et al., 1991; Quirion et al., 1992; Vilner and Bowen, 1992), although it can also possibly bind to the $\sigma 2$ site at concentrations comparable to that used in the present study (5 μ Ci, i.v.). Additional experiments using other σ -related radioligands such as 3 H-(+)pentazocine ($\sigma 1$) and 3 H-DTG (in presence of a saturating concentration of dextrallorphan) for $\sigma 2$ sites (DiPaolo et al., 1991; Bowen et al., 1992) will be required to clarify this issue. Already, in a series of preliminary experiments, we attempted to investigate the *in vivo* labeling of the mouse hippocampus following intravenous injections of either 3 H-DTG or 3 H-(+)pentazocine. However, in contrast to 3 H-(+)SKF 10,047, the amounts of radioactivity recovered in the hippocampal formation using either probe were too low to permit adequate pharmacological characterization (150–500 dpm per 5 μ Ci). Moreover, *in vivo* 3 H-DTG labeling was apparently mostly restricted to ventricular epithelia as revealed by *in vivo* autoradiography (P. Bouchard, Y. Dumont, A. Fournier, S. St-Pierre, and R. Quirion, unpublished observations). Thus, it appears that other radioligands will have to be developed in order to investigate adequately the *in vivo* binding profiles of $\sigma 1$ and $\sigma 2$ sites in the mouse brain.

The extent of the competition seen between 3 H-(+)SKF 10,047 and NPY-related peptides following their respective *in vivo* injections also supports the possible labeling by the radioligand of two classes of σ site. While haloperidol competed for up to 90% of 3 H-(+)SKF 10,047 labeling in the mouse hippocampus, intracerebroventricular injections of NPY and its homologs inhibited approximately one-third of the labeling, suggesting that 3 H-(+)SKF 10,047 could interact with subtypes of σ -sites, only one being accessible to NPY and related peptides.

The precise nature of *in vivo* interactions between 3 H-(+)SKF 10,047 labeling and NPY remains speculative. It clearly displays specificity as two other families of well-known peptides, neurotensin and VIP, failed to alter *in vivo* 3 H-(+)SKF 10,047 binding under our assay conditions. Similarly, the effects of NPY-related peptides are unlikely to be related only to changes in cerebral blood flow as adrenalin, a potent constrictor of the cerebral vasculature, as well as neurotensin and VIP, two peptides with potent cerebrovascular activities (Rioux et al., 1981; Wilson et al., 1981), failed to modify *in vivo* 3 H-(+)SKF 10,047 labeling of the mouse hippocampus.

We (Quirion et al., 1991) and others (Tam and Mitchell, 1991) were unable to obtain clear evidence for the existence of *in vitro* receptor binding interactions between a variety of σ - and NPY-related molecules. Interestingly, various peptidase inhibitors are generally used in *in vitro* binding assays to insure the integrity of NPY peptides used either as radioligands and/or competitors (Lynch et al., 1989; Martel et al., 1990a,b; Quirion et al., 1991). Could it be that an endogenously generated NPY metabolite is able to act as a σ ligand *in vivo*? While clear evidence is currently lacking to support this hypothesis, Contreras et al. (1987a) proposed the existence of endogenous σ ligands of peptidergic nature. It is thus of interest that only certain NPY-related peptides were able to modulate the *in vivo* labeling of 3 H-(+)SKF 10,047 in the mouse hippocampus. Both NPY-OH and the relatively selective Y_2 agonist NPY₁₃₋₃₆ (Wahlestedt et al., 1986) failed to demonstrate any activity in this assay. In contrast NPY, its homolog PYY, the fragment NPY₂₋₃₆, and the Y_1 agonist

Figure 4. Comparative effects of NPY ($n = 23$), neurotensin (NT; 1500 pmol, $n = 9$), VIP (1500 pmol, $n = 17$), and the catecholamine adrenalin (Ad; 1500 pmol, $n = 7$; 3000 pmol, $n = 11$) on *in vivo* 3 H-(+)SKF 10,047 labeling (total $n = 51$) in the mouse hippocampal formation. At this concentration, it is evident that only NPY significantly interacted with *in vivo* 3 H-(+)SKF 10,047 labeling. *, $p < 0.005$.

Leu³¹,Pro³⁴-NPY (Fuhlendorf et al., 1990) behaved as potent competitors. Taken together, these data may suggest that if a metabolite(s) of NPY is responsible for the inhibition of 3 H-(+)SKF 10,047/ σ labeling, it could be an N-terminal fragment likely containing amino acid residue located between positions 2 and 13. Alternatively, it could be that NPY and some of its related peptides can induce the release of a yet to be characterized endogenous σ ligand that could then inhibit the *in vivo* binding of 3 H-(+)SKF 10,047. Recent data by Connor and Chavkin (1991, 1992) are especially interesting in that regard as they provide evidence for the existence of endogenous σ ligands in the hippocampus. It is also possible that NPY-related peptides, by modulating signal transduction activity, could alter *in vivo* 3 H-(+)SKF 10,047/ σ labeling. These various possibilities are currently under investigation.

The ligand selectivity profile observed here for NPY and its homologs is most reminiscent of a Y_1 -like profile (Wahlestedt et al., 1986, 1990; Fuhlendorf et al., 1990; Quirion et al., 1990; Dumont et al., 1992) on the basis of the potent activity of the Y_1 analog Leu³¹,Pro³⁴-NPY and the ineffectiveness of NPY₁₃₋₃₆, a Y_2 agonist (Wahlestedt et al., 1986), to modulate hippocampal *in vivo* 3 H-(+)SKF 10,047 labeling. The high potency of PYY in this assay would also exclude an interaction with the newly characterized Y_3 receptor subtype as PYY was reported to be inactive on this receptor class (Balasubramaniam et al., 1990; Grundemar et al., 1991; Wahlestedt et al., 1992). While it is certainly too early to claim the existence of possible structural homologies between NPY and σ receptor classes, the pattern of *in vivo* interactions observed in the present study and those of others (Monnet et al., 1990a,b) raises interesting possibilities as the various NPY receptor subtypes (Rimland et al., 1991; Herzog et al., 1992; Larhammar et al., 1992), and likely the $\sigma 1$ site (Itzhak and Stein, 1991b; Itzhak et al., 1991; Bowen et al., 1992; Quirion et al., 1992) could all belong to the G-protein-coupled rhodopsin receptor superfamily.

In summary, the present study revealed the existence of *in vivo* interactions between certain NPY-related peptides and 3 H-(+)SKF 10,047/ σ labeling in the mouse hippocampus. Further studies are currently in progress to determine the mechanism(s)

responsible for these interactions and the possible functional significance of these findings.

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