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Phylogenetic appearance of Neuropeptide S precursor proteins in tetrapods

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Abstract

Sleep and emotional behavior are two hallmarks of vertebrate animal behavior, implying that specialized neuronal circuits and dedicated neurochemical messengers may have been developed during evolution to regulate such complex behaviors. Neuropeptide S (NPS) is a newly identified peptide transmitter that activates a typical G protein-coupled receptor. Central administration of NPS produces profound arousal, enhances wakefulness and suppresses all stages of sleep. In addition, NPS can alleviate behavioral responses to stress by producing anxiolytic-like effects. A bioinformatic analysis of current genome databases revealed that the NPS peptide precursor gene is present in all vertebrates with the exception of fish. A high level of sequence conservation, especially of aminoterminal structures was detected, indicating stringent requirements for agonist-induced receptor activation. Duplication of the NPS precursor gene was only found in one out of two marsupial species with sufficient genome coverage (*Monodelphis domestica*; opossum), indicating that the duplicated opossum NPS sequence might have arisen as an isolated event. Pharmacological analysis of both *Monodelphis* NPS peptides revealed that only the closely related NPS peptide retained agonistic activity at NPS receptors. The duplicated precursor might be either a pseudogene or could have evolved different receptor selectivity. Together, these data show that NPS is a relatively recent gene in vertebrate evolution whose appearance might coincide with its specialized physiological functions in terrestrial vertebrates.

Keywords

neuropeptide; evolution; tetrapods; arousal; sleep

1. Introduction

It is generally accepted that complex behaviors require complex neuronal substrates. It is also established that brain complexity increased during animal evolution and it can be assumed that anatomical complexity correlates with neurochemical diversity as well [22]. Thus, analyzing the phylogenic presence (or absence) of specific neurotransmitters can yield new insights into evolutionary “steps” of brain development and behaviors associated with it. In particular, the emergence of a new transmitter during evolution might also explain the acquisition of new physiological functions and behaviors that proved advantageous for the organism.

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We recently described that central administration of Neuropeptide S (NPS) is able to promote long lasting arousal in mice that is independent of environmental stimuli [23] and that NPS is produced in a previously uncharacterized group of neurons close to the locus coeruleus [24]. EEG recording in rats showed that NPS induces brain activity similar to enhanced wakefulness while suppressing all stages of sleep. In addition, we found that NPS produces an anxiolytic-like profile in behavioral tests that measure innate responses to unfamiliar or potentially dangerous environments in rodents. More recently, we demonstrated that NPS can restore sensory-motor gating in animal models of drug-induced psychosis-like behavior [15]. Such a pharmacological spectrum of behavioral effects is quite unique since classical stimulants such as amphetamine produce increased anxiety responses and aggravate psychotic-like behaviors. It is currently unknown if all these different behavioral effects of NPS are elicited simultaneously in vivo or particular functions are activated selectively upon specific environmental conditions. Also, it is unclear if NPS modulates similar behaviors in other species as all current behavioral data were obtained from rodents.

All vertebrate animals undergo some form of behavioral resting, followed by periods of increased activity, commonly referred to as sleep and wakefulness [14]. The transition from sleep to wakefulness requires some form of arousal and highest levels of arousal are usually correlated with situations of severe stress or physical activity. Most vertebrate species also display some form of emotional processing of sensory inputs. Those emotional responses commonly include fear and anxiety, leading to defensive behaviors that are vital for the survival of the organism. In order to study the evolution of complex behaviors such as arousal or anxiety it might be useful to follow the evolution of transmitter systems that are associated with them. Therefore, this study investigated the phylogenetic distribution of NPS precursor genes in currently available genomes. Determining the phylogenetic origin of NPS might help to understand the emergence of specific behavioral effects produced by NPS and shed new light on key events in vertebrate brain evolution.

2. Materials and Methods

2.1. Bioinformatic Data Mining and Analysis

Available genome databases were screened using BLAST algorithms with NPS cDNA and protein precursor sequences as queries. Databases included finished and unfinished genomes, whole genome shotgun sequencing (WGS) collections of unassembled sequences, expressed sequence tags (EST) and trace archives of ongoing sequencing projects. Similarity searches were performed online at the National Center for Biomedical Information (NCBI) from March 2005 until November 2006. Sequences with significant similarity to NPS or NPS precursor proteins were assembled, translated and analyzed using Lasergene version 7.0 (DNASar, Madison, WI). Alignments were made with MegAlign using the ClustalW algorithm.

2.2. Peptide Synthesis

Human, rat and mouse NPS peptides were custom synthesized by Sigma-Genosys (St. Louis, MO). Opossum NPS peptides were custom synthesized by Peptron Inc. (Busan, South Korea). All peptides were verified by mass spectrometry and purity was 80% or greater.

2.3. In Vitro Pharmacological Analysis

Pharmacological profiles of NPS peptides were obtained by measuring mobilization of intracellular free Ca^{2+} in cells stably expressing human or mouse NPS receptors (NPSR), as described before [18,23]. Briefly, cells were plated in 96-well plates 24 hours prior to the experiment and then loaded with Ca^{2+} -sensitive dye Fluo4-AM (Invitrogen, Carlsbad, CA) for one hour. After washing with assay buffer (Hank's balanced salt solution containing 0.1% bovine serum albumin; Sigma, St. Louis) peptides at different concentrations were added using

a pipetting robot and intracellular Ca^{2+} levels were monitored in a fluorimetric imaging plate reader (FLIPR, Molecular Devices, Sunnyvale, CA). Dose response curves were calculated with Prism software (GraphPad, San Diego, CA).

3. Results

3.1. Presence of NPS Precursor Sequences in Tetrapods

Sequences highly similar to human, rat or mouse NPS precursors were found in terrestrial vertebrate species including mammals, marsupials, birds, reptiles and amphibians. NPS-like sequences were identified from 29 species, including 6 primates, 3 rodents, 14 other mammals, 2 marsupials, 2 birds, 1 reptilian and 1 amphibian species (Fig. 1). Sequences with high homology to NPS precursor proteins were identified from 31 species that include all of the above plus two additional sequences that fell short of the part encoding the NPS peptide itself (*Dasypus novemcinctus* and *Spermophilus tridecemlineatus*; Supplementary Fig. 1). Notably, NPS-like structures were absent from fully or partially sequenced fish genomes, including the genomes of fugu (*Takifugu rubripes*), zebrafish (*Danio rerio*) and lamprey (*Petromyzon marinus*) that are highly covered. In addition, all available DNA data from four other fish species (*Gasterosteus aculeatus*, *Metriaclima zebra*, *Squalus acanthias* and *Tetraodon nigroviridis*) were analyzed without finding any evidence for sequences with sufficient similarity to NPS or NPS precursors. Also, NPS precursor proteins were not detected in genomic or EST sequences from invertebrates including insects, annelids, mollusks or cnidaria. From these data it can be concluded that during evolution NPS-like precursor genes might have first appeared in tetrapods, coinciding with the emergence of terrestrial vertebrates.

3.2. Phylogenetic Analysis of NPS Precursor Proteins

The genomic organization of NPS precursor genes appears to be identical in all species where sufficient sequence data were found: the gene is encoded by three small exons with a fairly large intron between the second and third exon. The start methionine and the second amino acid of the precursor protein are encoded by the presumed first exon. This exon could not be readily identified from genomic DNA data due to a lack of conservation in 5' untranslated regions. Only in cases of closely related species BLAST searches were sometimes able to detect the first exon (e.g. among primates). The second exon always encodes the signal peptide and the third exon contains the remaining protein including the NPS sequence, followed by 3' untranslated regions. In most cases, homologous DNA sequences were found for the second and third exon and precursor proteins were deduced from there. Table 1 contains a list of the GenBank DNA sources that were used to identify and deduce the NPS precursor sequences discussed in this paper. Alignment of deduced NPS precursor proteins shows that the highest degree of homology is found within the signal peptide, the presumed proteolytic cleavage site immediately preceding the NPS sequence and the NPS coding sequence itself (Supplementary Fig. 1). Conversely, the spacer region between the signal peptide and immature NPS displays little conservation across species. Phylogenetic analysis shows that precursor proteins from related species also display the highest degrees of homology. For example, primate NPS precursor proteins including human, chimpanzee, orang-utan, marmoset, and macaque cluster on the same branch of the phylogenetic tree (Fig. 2). Some inconsistent branching of the phylogenetic tree is mainly caused by the variable length and relatively small size of available NPS precursor sequences (for example, from 11 species only exon 3 sequences were identified). Overall protein identities vary widely, ranging from 93% amino acid identity between human and chimpanzee to only 35% between zebra finch and human NPS precursors.

3.3. Conservation of the NPS Peptide Sequence

The NPS peptide coding sequence has been conserved at a high degree. Only a few positions in the center and carboxyterminal part of the peptide show sequence variation, indicating that

the aminoterminal might contain critical structures for receptor binding and activation. The first seven amino acids of NPS are completely conserved among all species analyzed (Fig. 1). Position 8 shows only conservative amino acid substitutions from the most common threonine to serine, methionine, alanine or glutamine. In contrast, position 10 appears to allow for more flexibility as various hydrophobic or neutral amino acids (Met, Ile, Val, Leu, Ala, Thr, Gly) are found in this position, with Met being the predominant allele. A pair of lysine residues is found at position 11 and 12 in all species with only two exceptions. Bottlenose dolphin NPS contains an Arg-Asn sequence in this position while the sequence derived from macaque monkey deviates from the common lysine in position 12 by harboring an asparagine residue in this place. Similarly, threonine 13 is perfectly conserved among all species, only *Xenopus* NPS displays a substitution to asparagine. Serine¹⁴ is common to all NPS sequences, except for the bat NPS sequence that contains a proline in this position. Human, galago, elephant, horse, hyrax, European shrew and dolphin NPS are the only sequences containing a glutamine residue at position 15 instead of the common arginine found in all other species. The penultimate lysine residue is highly conserved, only anole NPS carries a conservative substitution to arginine in this position. Finally, the carboxyterminal amino acid position displays considerable variation (Ser, Leu, Gln, Pro) with the majority of NPS peptides containing a serine residue in this position. Only horse, elephant and rock hyrax NPS sequences are identical to human NPS. Analysis at the DNA level revealed that most sequence variations can be explained by single nucleotide exchanges, indicating that the NPS precursor protein may have arisen from a single ancestral gene.

3.4. Gene Duplication in Opossum

The NPS precursor gene appears to exist as a single copy in all genomes analyzed with the exception of the marsupial *Monodelphis domestica* (opossum). Searching for distantly related sequences, two genomic opossum sequences were found that show modest sequence similarity to other NPS precursors and to each other. The opossum genome encodes one typical NPS precursor protein and the deduced opossum NPS peptide sequence contains only two amino acid substitutions compared to human NPS (Fig. 1). This precursor sequence will be referred to as “*opossum prepro-NPS I*” in this manuscript and the encoded peptide as *opNPS I*, accordingly. Synonymously, the second related opossum sequence will be termed *opossum prepro-NPS II* and *opNPS II*, respectively. Opossum prepro-NPS I displays the same intron-exon architecture as NPS precursors from other species, containing a large intron between the exon encoding the presumed signal peptide and the downstream exon encoding immature NPS (note that the presumed first exon could not be identified from genomic DNA sequences due to the low sequence conservation of the 5' untranslated region). The deduced protein contains an aminoterminal signal peptide, followed by a spacer region and an NPS-like sequence at the carboxyterminus. In contrast, the opossum prepro-NPS II genomic sequence does not contain an intron between the presumed signal peptide and the carboxyterminal part of the precursor, but appears to be intronless. A potential translational start site could not be identified due to the lack of conservation in exon 1 of the NPS precursor gene. The NPS-like peptide in opossum prepro-NPS II is preceded by a potential proteolytic cleavage site (Lys-Arg), similar to the common NPS precursor (Fig. 3). The deduced NPS-like peptide (termed *opNPS II*), however, shows only a low degree of similarity with *opNPS I* or NPS peptide sequences from other species. Only the first five amino acids of *opNPS II* show significant homology to the common NPS sequence. The canonical arginine in position 3 has been replaced by a histidine residue in *opNPS II*, thus still preserving a potential positive charge at this position. The central part of *opNPS II* contains an insertion of two amino acids and a negatively charged glutamate residue that is absent from any other NPS peptide structure. Interestingly, *opNPS II* shows high sequence homology with other NPS peptides in the carboxyterminal half following the common Lys-Lys motif, only Arg¹⁶ has been replaced by glutamate. Amino acid identity

between *opNPS* I and *opNPS* II is 65% while the putative precursor proteins share 73% amino acid identity and 82% overall similarity.

3.5. Functional Analysis of Opossum NPS Peptides

From the current data it is unclear whether both NPS precursor-like sequences in the opossum genome are transcribed and translated or whether opossum prepro-NPS II is merely a pseudogene. In order to address this question in a functional way, the agonistic potencies of both peptides were tested in transfected cells that stably express NPS receptors. The low level of sequence similarity of *opNPS* II with other NPS peptides raises the question whether *opNPS* II is still able to activate NPS receptors or may have its own pharmacologically distinct target. As shown in Fig. 4, only *opNPS* I behaved as a full agonist at human and mouse NPS receptors. While *opNPS* I induced Ca^{2+} mobilization in cells expressing mouse NPSR with an EC_{50} value of 4.1 ± 1.2 nM, *opNPS* II was completely inactive at concentrations up to 10 μM . The EC_{50} of *opNPS* I at human and mouse NPS receptors is very similar to those found with other mammalian NPS peptides [18].

4. Discussion

4.1. NPS and the Evolution of Tetrapods

The primary structures of NPS peptides are highly conserved, indicating strong evolutionary pressure or a relatively recent origin of the gene. NPS precursor-like sequences are absent from fish, thus NPS may have originated relatively late during vertebrate evolution and may coincide with the emergence of tetrapods. Fossil and DNA records indicate that the split between ancient fish and tetrapods occurred around 350 million years ago [9]. NPS precursor genes were found in one amphibian (*Xenopus*) and one reptilian (*Anolis*) genome, representing early branches of tetrapod evolution. Database searches using the *Xenopus* or *Anolis* NPS precursor sequences as query against currently available fish genomes could also not detect any NPS-like sequences. Clearly, NPS precursor-like sequences are absent from Actinopterygii (ray-finned fishes) as there are extensive genomic DNA data from multiple species available in this taxonomic class. On the other hand, genomes of coelacanth or lung fishes, that are also referred to as lobe-finned fishes and together with the tetrapods form the class of Sarcopterygii, have not yet been sequenced in detail. It is therefore unclear whether or not NPS-like peptides exist in lobe-finned fishes that are presumed to be the closest living relatives to tetrapods [25]. From the currently available data it can therefore be concluded that NPS precursor-like sequences are specific to tetrapods, including mammals, birds, reptiles and amphibia.

The evolution of bioactive – or more specifically: neuroactive – peptides in metazoans has been progressive and complex. There are only a few examples of bioactive peptides conserved across the entire animal kingdom. Peptides similar to insulin or insulin-like growth factors are found from invertebrates to mammals [20,12]. Neuropeptides with the carboxyterminus Arg-Phe-amide (RFamides) are also found from cnidaria [17] to human [3], although their structural diversity implies multiple genetic origins [8]. With rising complexity in their nervous systems, vertebrates appear to have evolved a large number of neuropeptide genes that do not have counterparts in invertebrates. The families of endogenous opioid peptides [5], NPY-like peptides [4], orexin/hypocretin-like peptides [1] or CRF/urocortin-like peptides [10] occur in all vertebrates including ray-finned fishes. It appears that preproNPS is so far the only example of a peptide precursor that is absent from fish but is present in all tetrapod vertebrates.

4.2. Possible Functional Implications

One possible functional correlate for the restricted occurrence of NPS in tetrapods could be the development of highly structured sleep-wake cycles in terrestrial vertebrates. We have demonstrated previously, that NPS induces profound wakefulness and suppresses all stages of

sleep in rodents [23]. Although fish also display periods of behavioral resting and other sleep-like physiological parameters [26], it is obvious that their physically unstable environment (i.e. moving waters) will not allow for mammalian-like sleep with loss of consciousness and reduced muscle tone. Mammalian-like sleep, that includes loss of consciousness and voluntary motor control, would be detrimental to a species living in moving waters and could only be useful for a land-based animal. On the other hand, the evolutionary development of sleep also necessitates the development of neuronal systems that induce wakefulness or maintain an awakened state. Two other neuropeptides that are prominently involved in the regulation of sleep/wake states in mammals are the orexins/hypocretins [21]. It is interesting to note that a recent study in goldfish brain found anatomical evidence that the orexin/hypocretin system might not be involved in the regulation of wakefulness but is more likely modulating energy homeostasis in fish [6]. However, another study found hypolocomotor effects induced by orexin/hypocretin in zebrafish, which could be a correlate of behavioral arousal [13], similar to the effects of orexin/hypocretin in mammals. Obviously, further studies into the neurophysiological and neurochemical basis of sleep-like behavior in fish are necessary. In contrast to fish, aquatic mammals like dolphins have developed unihemispheric sleep, allowing them to control motion and body posture during sleep [16,11]. This specialized form of sleep still requires awakening of the sleeping brain hemisphere and NPS might be involved in this process. It is commonly accepted that dolphins and whales developed from previously terrestrial mammals and it is therefore not surprising that the bottlenose dolphin NPS precursor shares high homology with NPS precursors from elephant, cattle, pig or dog. We have also shown previously that NPS induces anxiolytic-like effects in tests of fear or natural defensive behavior [23]. Although anxiety-like and stress-related behaviors are also documented in fish [7], it is possible that the NPS system has attained specialized functions to modulate certain aspects of emotional states in tetrapods. Clearly, these hypotheses reward further research about the differences in neural circuits regulating arousal and anxiety between fish and tetrapod vertebrates. Comparative anatomical analysis of NPS precursor and receptor expression in various species will be necessary to evaluate whether or not the NPS system might serve similar physiological functions across tetrapod vertebrates.

4.3. Evolution of NPS Peptides Confirms Structure-Activity Studies

The evolutionary conservation of the NPS peptide sequence indicates that aminoterminal structures are most important for biological activity of the peptide. Indeed, we have shown before that an aminoterminal deletion in the NPS peptide completely abolishes its ability to activate NPSR while deletions or changes in the carboxyterminal part of the peptide have no or little effect [18]. These findings were later confirmed by others [19,2]. The perfect conservation of the first seven amino acids of NPS across all species clearly reflects the structural constraints for biological activity. The duplication of NPS precursors in opossum is most likely a unique event since no duplicated NPS precursor-like sequence could be found in a second marsupial, *Macropus eugenii*. The intronless structure of opossum preproNPS II, the mutation of critical aminoterminal residues in *opNPS II*, and most importantly the lack of activity of *opNPS II* indicate that this sequence might be a pseudogene without selective evolutionary pressure. Alternatively, *opNPS II* might have developed a different receptor selectivity, although this possibility appears rather unlikely since only one genomic sequence with high similarity to NPSR could be found in the available opossum genome (data not shown).

4.4. Summary

In summary, this study provides evidence that the occurrence of DNA sequences encoding NPS precursor proteins and peptides coincides with the evolution of tetrapods and might therefore indicate specialized functions that are required in tetrapod nervous systems but are absent or carried out by different neurotransmitters in fish. Studies of the physiological effects

of NPS in various tetrapod vertebrates will be necessary to address the question whether NPS has common functions in all tetrapods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

EST	expressed sequence tag
NPS	neuropeptide S
NPSR	neuropeptide S receptor

SFRNGVGTGMKKTSFQRAKS	Homo sapiens (human)	
SFRNGVGTGMKKTSFRRRAKS	Pan troglodytes (chimpanzee)	
SFRNGVGTGMKKTSFRRRAKS	Pongo pygmaeus (orang-utan)	Primates
SFRNGVGTGMKKTSFRRRAKS	Callithrix jacchus (marmoset)	
SFRNGVGTGGKKTSFQRAKS	Otolemur garnettii (galago)	
SFRNGVGTGMKNTSFRRRAKS	Macaca mulatta (macaque)	
SFRNGVGTGMKKTSFPRAKP	Cavia porcellus (guinea pig)	
SFRNGVGSQAKKTSFRRRAKQ	Mus musculus (mouse)	Rodents
SFRNGVGSQVKKTSFRRRAKQ	Rattus norvegicus (rat)	
SFRNGVGTGMKKTSFRRRAKS	Bos taurus (cattle)	
SFRNGVGTGMKKTSFRRRAKS	Canis familiaris (dog)	
SFRNGVGTGMKKTSFQRAKS	Equus caballus (horse)	
SFRNGVGTGMKKTSFRRRAKS	Echinops telfairi (small Madagascar hedgehog)	
SFRNGVGAGTKKTSFRRRAKS	Erinaceus europaeus (western European hedgehog)	
SFRNGVGTGMKKTSFQRAKS	Loxodonta africana (African elephant)	
SFRNGVGTGLKKTSFPRAKS	Myotis lucifugus (little brown bat)	
SFRNGVGTGMKKTSFRRRAKL	Oryctolagus cuniculus (rabbit)	Other Mammals
SFRNGVGTGMKKTSFQRAKS	Procavia capensis (rock hyrax)	
SFRNGVGMGMKKTSFQRAKS	Sorex araneus (European shrew)	
SFRNGVGTGMKKTSFRRRAKP	Sus scrofa (pig)	
SFRNGVGTGMKKTSFRRRAKS	Tupaia belangeri (northern tree shrew)	
SFRNGVGTGMKNTSFQRAKS	Tursiops truncatus (bottlenose dolphin)	
SFRNGVGQGIKKTSFRRRAKS	Ornithorhynchus anatinus (platypus)	Egg-laying Mammals
SFRNGVGTGIKKTSFRRRAKS	Macropus eugenii (wallaby)	Marsupials
SFRNGVGSQMKKTSFRRRAKS	Monodelphis domestica (opossum)	
SFRNGVGSQGIKKTSFRRRAKS	Gallus gallus (chicken)	Birds
SFRNGVGAGIKKTSFRRRAKP	Taeniopygia guttata (zebra finch)	
SFRNGVGSQMKKTSFRRRAKL	Anolis carolinensis (green anole)	Reptiles
SFRNGVGSQGIKKNSFRRRAKL	Xenopus tropicalis (western clawed frog)	Amphibia

Figure 1.

Alignment of NPS peptide sequences deduced from DNA sequences as described in Table 1. Amino acid residues are given in single letter code and black shaded letters indicate differences from the human NPS sequence.

Figure 2.

Phylogenetic tree of NPS precursor protein sequences deduced from GenBank DNA resources as described in Table 1. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of nucleotide substitution events. Deviations from common phylogenetic models might be caused by unequal length of the aligned sequences. Calculations were performed using the ClustalW algorithm in MegAline, Lasergene version 7.

SSVRLNLFVLWISSMRMLCCYPTPSAMVSEKSDYFLILLNSCLAAMDRSEELAF LKPFLVKNAMKRSFRNGVGSGM--KKTSFRRAKS	op NPS I
SS+RLNLFVLW SS +ML CYPTPS M+S+K DYFLILLNSCLAA R+EEL FLK FL+KN MKRSF NG+ G+ KKTSE RAKS	
SSLRLNLFVLWTSSPQMLYCYPTPSFMLS KKF DYFLILLNSCLAAKGRNEELLFLKLFL LKNVMKRSFHNGIEIGI IHKTSFERAKS	op NPS II

Figure 3.

Alignment of the two NPS precursor-like proteins deduced from opossum genomic DNA resources. The middle line depicts identical amino acids while “+”-signs indicate conservative substitutions. Dashes (–) were added to optimize the alignment.

Figure 4. Pharmacological activity of *opNPS I* (■) and *opNPS II* (▲) at mouse NPSR stably expressed in HEK 293 cells. Incubations were done in triplicate and repeated twice with similar results. Data from a representative experiment are shown as means $\pm$ SEM. EC₅₀ values were calculated from fitted curves using nonlinear regression analysis.

Table 1

DNA Sources

Species	Chromosome	cDNA/EST (GB access. #)	Genomic contig (GB access. #)	Genomic trace (TI #)
<i>Anolis carolinensis</i>				1344121498
<i>Bos taurus</i>	26	DN276816	NC_007327, AAFC03054291	
<i>Callithrix jacchus</i>				1056145563, 1056050229
<i>Canis familiaris</i>	28		NC_006610, AAEX02015323, AACN010688913	
<i>Cavia porcellus</i>			AAKN01631318	
<i>Dasyurus novemcinctus</i>			AAGV01197291, AAGV01197292	
<i>Echinops telfairi</i>				709150592
<i>Equus caballus</i>				1225188001, 1237961856
<i>Erinaceus europaeus</i>			AANN01154424	
<i>Gallus gallus</i>	6	BU293859	NW_068630, AADN02047072	253783316
<i>Homo sapiens</i>	10q25	NM_001030013	NC_000010, AL355316	
<i>Loxodonta africana</i>			AAGU01366225, AAGU01366222	
<i>Macaca mulatta</i>	9		NC_007866, AANU01120863	
<i>Macropus eugenii</i>				976321090
<i>Monodelphis domestica</i>			AAFR03061136	
<i>Mus musculus</i>	7	BD438828	NC_000073, AC131669	
<i>Myotis lucifugus</i>				981598217
<i>Ornithorhynchus anatinus</i>				1010427631
<i>Oryctolagus cuniculus</i>			AAGW01227495	
<i>Otolemur garnettii</i>			AAQR01586866	
<i>Pan troglodytes</i>	10		NC_006477, AACZ02118543	
<i>Pongo pygmaeus</i>				721196182, 757158301
<i>Procavia capensis</i>				1220395683
<i>Rattus norvegicus</i>	1	BD438850	NC_005100, AAHX01008831	
<i>Sorex araneus</i>			AALT01023589	
<i>Spermophilus tridecemlineatus</i>			AAQQ01627670	
<i>Sus scrofa</i>				1420055967
<i>Taeniopygia guttata</i>				1400029257
<i>Tupaia belangeri</i>			AAPY01217820, AAPY01661898	
<i>Tursiops truncatus</i>				1479713402, 1485868060
<i>Xenopus tropicalis</i>				422934897

GenBank accession numbers and chromosome location (where available) of NPS precursor-like sequences. Species are listed alphabetically.