

AMINO Vol. II — The First Commits

every signal molecule you share with the animals, and when
nature wrote its code

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11 chapters · single-language edition

Every number, name and year in this volume is traceable to a cited source.

01. One Morning, Every Molecule

The alarm sounds at 6:12, and the first thing that happens is a changing of the guard. Through the night, a small knot of neurons at the base of your brain has been held quiet; now it comes up firing, and the molecule it broadcasts — histamine, the same one that will torment hay-fever sufferers all afternoon — switches the cortex from idle to awake. The night shift stands down with it: GABA, the brain's word for no, loosens its hold as the waking circuits shrug it off. You surface. You are conscious for four seconds before the first want of the day arrives, and the want is coffee — not the taste of it, which is an hour away, but the pull toward it, which is dopamine's department and available immediately.

The morning continues in chemistry. You did not wake once in the night to use the bathroom, because vasopressin spent those hours telling your kidneys to hold water — the unglamorous day job of a molecule that will spend this book's eighth chapter tangled in claims about love. Shaving, you nick your chin, and the bleeding stops in under a minute: platelets that never manufactured a molecule of serotonin in their lives arrive carrying it anyway, and fire it into the wound to close the vessels. Breakfast is rice porridge with a spoon of fish sauce, and the deep, mouth-filling savour of it is glutamate pressing on receptors your tongue keeps for exactly this — the most abundant signalling molecule in your brain, met first as a taste. On the ride to work a delivery truck runs the light, and before any part of you has decided anything your heart rate has doubled and your pupils have gone wide: noradrenaline down the sympathetic chain, adrenaline from the adrenal glands riding behind it. At the office gate you sneeze, twice — histamine again, this time released by immune cells meeting pollen, working its other, older-seeming job. Someone you love texted while you were riding; reading it, something eases in your chest, and folk wisdom will tell you the something is oxytocin, a claim this book will weigh rather than repeat. By the time you sit down, the toe you stubbed on the bed frame has stopped hurting,

which is at least partly the work of peptides that behave like the body's own morphine — partly being a word chapter nine will insist on.

One ordinary morning. Eight families of molecules, every one of them older than your species, older than mammals, older — most of them — than animals with heads. That is the subject of this book. Not one of these messengers is human. You share glutamate with a vine reacting to a caterpillar bite, serotonin with creatures that have no brain to be happy in, dopamine with a water flea, the bonding peptides with an earthworm, and the machinery of the adrenaline rush with every fish that ever startled. The inventory is shared because it was inherited, and it was inherited because it was written early and never rewritten.

Nature as a codebase

This book reads that inventory the way an engineer reads inherited code. The metaphor is the book's owner's own, and it earns its keep because it asks the right question. Confronted with a working system nobody documented, an engineer does not ask what is this? — she asks when was this written, what was it written for, and why has nobody dared to change it? Every module in a living cell has an answer to those questions. The answer is called its history, and for the molecules of this book, that history is recoverable: written in gene trees, in the chromosomal neighbourhoods that duplication leaves behind, in which living lineages carry a working copy and which never received one. The question every chapter asks is the engineer's: how did nature write this code — and when was the first commit?

First commit needs saying precisely, because it hides four different questions. A molecule can exist as a metabolite — a by-product of the chemistry of being alive — long before anything listens to it. Something can listen — a receptor can exist — before there is a nervous system to wire it into. It can be wired into circuits long before it acquires the job that made it famous. Serotonin the wound-closer, serotonin the gut-mover and serotonin the target of antidepressants are three different functions with three different birthdays, and flattening them into

"serotonin is X million years old" is the error this book is built to avoid. So each chapter ends in a **Commit Ledger**: one row per function, never one row per molecule.

A ledger row looks like this — two real ones, borrowed from later chapters:

| Function | First commit | Grade | What would move the date |

|---|---|---|---|

| Histidine biosynthesis (histamine's precursor pathway) | Pre-LUCA, ~3.5–4 Gya — comparative genomics of the his genes | A | A redating of LUCA; a domain building histidine another way |

| Human trust / social cognition (oxytocin) | Founding result 2005; critical review 2015; registered replication 2020; no robust effect | D | A large pre-registered trial with confirmed central delivery |

Read the columns left to right. The function, kept narrow. The first commit as a range with its method named — molecular clock, fossil calibration, comparative genomics, structural inference — because a date without a method is a rumour, and the methods disagree often enough that the disagreement itself gets printed; and where a function cannot honestly be dated at all, the ledger prints undated or undatable rather than inventing a number. The grade: **A** means at least two independent lines of evidence converge; **B**, one solid line; **C**, a single contested line or a preprint; **D**, an informed inference wearing its uncertainty openly. And the last column is the book's handshake with its own mortality: the specific finding that would rewrite the row. Between those two sample rows runs the whole span of this book — a pathway older than the last universal common ancestor graded A, and a headline about human nature, seventy times younger, graded D. Age and certainty travel together here more often than you might expect, and chapter ten will make something of that.

Behind every ledger stands one more question, the deepest in the book: why these molecules? Three answers compete. The **frozen accident** says

the assignments were arbitrary and then became permanent — once a thousand other systems depended on serotonin meaning what it means, the cost of switching became infinite, and the accident froze; the phrase comes from Francis Crick, whose original argument about the genetic code Vol. I told in full. **Selection** says the molecules are still here because they kept winning — tested against alternatives for six hundred million years and never beaten. **Chemical constraint** says there was never a real choice: the cast was whatever the chemistry of amino acids offered cheaply, and evolution shopped in the only store that was open. The three make different predictions, they can be told apart by evidence, and the final chapter will hold each family up against all three rather than crowning one in advance.

A word about the volume in your hands versus the one before it. Vol. I began at the beginning — the first handed molecule, the genetic code, the RNA world — and descended to LUCA, the last universal common ancestor, a population it left dated at about 4.2 billion years old with a 95 percent credible interval of 4.09 to 4.33 Gya. Those anchors hold this book up, and when a chapter here needs them it will cite them rather than re-derive them. But this volume walks the other way. Vol. I started at the origin and came forward; this book starts in your morning and goes back. Where Vol. I already told a story — a lobster raised on serotonin, a water flea running dopamine's chemistry in its gut, a frog retuned by an injected peptide — you will get one clause and a pointer, never a retelling. The scenes of your morning work the same way: each is an advance on a chapter, and each chapter pays its scene's sources in full.

The first word to trace is the one you tasted at breakfast — the workhorse your cortex is using to read this sentence, which chemistry put to work almost four billion years before anything could read at all.

Overtaken if: the shared inventory itself proves shallower than it looks — if the receptor families this book dates to deep common ancestors keep resolving, on closer genomic reading, into lineage-by-lineage reinventions, the way chapter seven's control-group molecule already has.

Sources

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- **2026** · AMINO: The Complete Book (Vol. I), chs. 01-14 — the anchor dates and told stories this volume references throughout are sourced there
- All morning-scene claims are advances on chapters 02-09 and carry their full sourcing in those chapters' ledgers.

02. Glutamate: The Oldest Word

In a laboratory at Tokyo Imperial University in 1907, the chemist Kikunae Ikeda is boiling seaweed. The stock is konbu dashi, the kelp broth beneath most of Japanese cooking, and Ikeda has decided its taste is none of the four his textbooks allow. Over months he works through some ninety pounds of kelp, stripping out salts and sugars a fraction at a time, until about an ounce of brown crystals is left. He tastes them: not sweet, sour, salty or bitter, but a fifth thing, savoury and mouth-filling and slow to leave, and he names it umami. The crystals were glutamic acid. A patent followed in April 1908, granted that July; by 1909 the Ajinomoto company was making monosodium glutamate by the ton.

Ikeda's own paper dropped out of the international literature, surviving in English only as a three-quarter-length translation published ninety-three years later, beside Bernd Lindemann's account of its reception. The mechanism took nearly as long. In 2002 Greg Nelson, Nicholas Ryba, Charles Zuker and colleagues reported the receptor in *Nature*: T1R1+T1R3, a G-protein-coupled receptor — a protein threaded through the membrane that binds something outside the cell and starts a cascade inside — built from two subunits and tuned broadly to L-amino acids, glutamate strongest among them. Ikeda had not invented a taste. He had found a molecule the tongue was already built to notice.

That receptor is the newest thing in this chapter. Glutamate is not, at bottom, a messenger: it is a metabolite, a working chemical of ordinary cell chemistry, made and consumed by cells with no interest in signalling anyone. Glutamate dehydrogenase, present in all three domains of life, runs $\text{glutamate} \rightleftharpoons \alpha\text{-ketoglutarate} + \text{ammonia}$ — the hinge between amino-acid nitrogen and the carbon cycle that burns sugar. Around it sit the aminotransferases, which lift an amino group off one carbon skeleton onto another; comparative genomics places their divergence into four classes before LUCA, roughly four billion years ago. Because they funnel amino groups from most other amino acids through the glutamate/ α -

ketoglutarate pair, glutamate is not merely abundant. Every cell that handles nitrogen handles glutamate.

Its recognition as a neurotransmitter lagged for decades, and the ubiquity was the reason: a signal was expected to be scarce and dedicated, not the cell's nitrogen currency at five to fifteen millimoles per kilogram of brain tissue. What was missed was most of the brain. Counting cortical excitatory synapses as glutamatergic, David Attwell and Simon Laughlin's 2001 energy budget for grey matter puts roughly ninety percent of synapses in that class, from anatomical counts of 1991 and 1998; a stricter denominator gives thirty to forty percent. Excitation is the default, and the default has no brake of its own.

Traced backward, the receptor leaves the animals almost at once. In 1999 Guo-Qiang Chen, Changhai Cui, Mark Mayer and Eric Gouaux described GluR0 in *Nature*, from the cyanobacterium *Synechocystis* PCC 6803 – the first glutamate receptor found in a prokaryote. It is ionotropic, meaning the ligand's binding opens a pore directly rather than starting a chemical relay, and it reads as a bacterial amino-acid-binding protein bolted onto a potassium channel. What it is not is evidence that bacteria talk to one another in glutamate: GluR0 has no known cell-to-cell role; nutrient sensing is likelier. The fold is ancient; the conversation is not.

Plants took the same fold somewhere else. In 2018 Masatsugu Toyota and colleagues reported in *Science* that wounding one leaf of *Arabidopsis* releases glutamate from the damaged cells, and a wave of calcium travels through the plant's plumbing to distant, untouched leaves within minutes, switching on defence genes there. The sensors are GLUTAMATE RECEPTOR-LIKE proteins, homologs of the animal channel, running alarm traffic in an organism with no neurons and no synapses. Where that family came from is unsettled: one comparative-genomics line places an ancestral GLR in algae with continuous descent into land plants, another finds land-plant GLRs unrelated to green-algal ones. The mechanism is dated to the year. The lineage is not.

The sharpest result belongs to the other receptor line. In 2006 Donghui Kuang, David Hampson, Belinda Chang and colleagues reconstructed the

ancestral ligand-binding pocket of the Family C G-protein-coupled receptors — the class the metabotropic glutamate receptors belong to, metabotropic meaning the receptor starts a cascade rather than opening a pore. Ancestral reconstruction infers an extinct protein's sequence from living descendants, builds it, and tests it. The resurrected pocket already bound L-glutamate with high affinity, at a node long predating any synapse, and that line roots in single-celled eukaryotes. The sensor came before the use.

Inside the animals the ionotropic channels radiate. Àlex Ramos-Vicente and colleagues resolved that radiation in eLife in 2018 into four subfamilies — AKDF, Epsilon, Lambda and NMDA — established within the animal stem, which Vol. I's Bayesian clock brackets between 649.8 and 833.5 million years ago. As Vol. I told through the sponge's empty postsynaptic scaffold and the ctenophore's glutamate-only nervous system, the parts of a synapse do not arrive as a set. Placozoans sharpen that: *Trichoplax adhaerens* and its relatives have no neurons, no synapses and no nervous system, yet carry working Epsilon- and AKDF-class channels while lacking NMDA outright — and their one characterized Epsilon receptor is not gated by glutamate at all. Glycine, alanine, serine and valine open it, and three substitutions flip its preference to glutamate, as Singh, Bowie and Senatore showed in 2025. Vol. I found scaffold without receptor. This is receptor without a nervous system, and without glutamate.

The Word the Family Did Not Start With

Which of those events, then, is the first glutamate signal? The question is harder than it looks, because the family's name came from its founding vertebrate members rather than from its ancestral ligand. In 2010 Vincent Croset, Richard Benton and colleagues showed in PLOS Genetics that a whole divergent branch of it, the protostome Ionotropic Receptors, was repurposed as the molecular basis of insect smell and taste: same fold, synaptic job abandoned, scaffold turned outward. Ramos-Vicente's own tree holds an amphioxus receptor gated by glycine. Kuang and Chang's

resurrection reads as a pocket glutamate-shaped from the beginning; the redeployments read as a generic amino-acid detector on which glutamate is one setting among several. Neither frames its work as a rebuttal of the other; the disagreement sits in the data, not between the people.

The same discipline applies to function. Vertebrate NMDA receptors are coincidence detectors: a magnesium ion plugs the pore and is expelled only when the cell is already depolarized and glutamate is bound, so current passes only when two conditions coincide — the substrate for Hebbian learning, the rule that neurons firing together strengthen their connection. That property is not the family's birthright. In the fruit fly's dNR2 subunit the asparagine residue governing the block is a glutamine instead, and *C. elegans* NMDA-type receptors may lack it altogether, plausibly tracking the higher magnesium those animals carry. Coincidence detection looks like a vertebrate-side refinement laid over an older, blunter channel, and no ancestral reconstruction of that site exists to date it. The class runs at least as deep as the cnidarian-bilaterian ancestor, yet no vesicular, synapse-specific release of glutamate has ever been confirmed in a cnidarian. Receptor existence and synaptic use are two different commits.

The oldest rows in this log are its firmest and the most familiar one is its weakest. Glutamate's place at the centre of nitrogen metabolism rests on comparative genomics across all three domains; the receptor radiation on a published clock with stated bounds; the property that makes a vertebrate synapse capable of learning carries no date at all, filed at grade D for that reason. What the trace agrees on is the ordering — before the synapse a receptor, before the receptor a metabolite, and the receptor, when it appeared, not yet particular about which amino acid it answered to. What Ikeda tasted in 1907 was the oldest word in the vocabulary, and a vocabulary whose only word means more is not yet a language: the next commit is the one that learned to say no.

Commit Ledger

| Function | First commit | Grade | What would move the date |

|---|---|---|---|

| Metabolic nitrogen hub (glutamate/ α -ketoglutarate transamination) | ~4 Gya, pre-LUCA – comparative genomics of four aminotransferase classes (Koper 2022; Smith 2017) | B | An independent structural-clock or isotopic study corroborating the pre-LUCA divergence |

| Ionotropic channel scaffold exists | Predates the bacteria/eukaryote split, node unresolved – homology inference from one bacterial exemplar (Chen 1999) | C | A second prokaryotic or archaeal channel of the same fold |

| Ionotropic family radiates into four subfamilies | Animal stem, bounded by crown Metazoa at 649.8–833.5 Mya – Bayesian clock (dos Reis 2015) on a gene-tree phylogeny (Ramos-Vicente 2018) | B | A resolved sponge-versus-ctenophore-first answer |

| NMDA-class receptor exists | By the cnidarian-bilaterian ancestor, bounded by crown Eumetazoa at 625.9–746.2 Mya – Bayesian clock (dos Reis 2015); absent in placozoans, sponges, ctenophores (Singh 2025) | B | An NMDA-class gene confirmed in a sponge or placozoan genome |

| NMDA coincidence detection (magnesium block) | Undated – comparative pharmacology only; strong in vertebrates, weak or absent in *Drosophila* and *C. elegans* (Ramos-Vicente 2018) | D | An ancestral-sequence reconstruction of the magnesium-block residue across the animal tree |

| Vesicular packaging and quantal release (VGLUT) | To the protostome-deuterostome floor – rocks-versus-clocks: >600 Mya conventional, 642–761 Mya clock, ~558–575 Mya fossil; confirmed in *C. elegans* (Serrano-Saiz 2020; dos Santos 2025) | B | A functional VGLUT ortholog confirmed in a cnidarian or sponge |

| Glutamate as the dominant fast excitatory transmitter | Coincident with the radiation above – gene-tree phylogeny plus Vol. I's ctenophore genomes (Ramos-Vicente 2018) | B | Confirmed vesicular glutamate release, not receptor responsiveness, in a cnidarian |

| Plant long-distance wound signalling via GLRs | Mechanism dated 2018 by calcium imaging; family origin contested – continuous algal descent versus no close algal relation (Toyota 2018) | C | A resolved algae-to-land-plant GLR phylogeny |

| Human cortical synaptic dominance (~90%; ~30–40% on a stricter denominator) | Modern measurement, not a deep-time date – anatomical synapse counting (Attwell & Laughlin 2001) | B | A standardized whole-brain connectomic count superseding the 1991 and 1998 estimates |

Overtaken if: an ancestral-sequence reconstruction across the animal ionotropic tree shows the ancestral channel was already glutamate-selective, making the placozoan receptor's taste for glycine, alanine, serine and valine a late local quirk rather than a survival of the original state.

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03. GABA and Glycine: The Invention of No

The anaesthetist asks you to count backward from ten. Ten is clear. Nine is clear. Somewhere near eight the edges of the room soften, the sentence you were assembling loses its verb, and seven never arrives – not because you declined it, but because the part of you that would have said it is no longer permitted to speak. From outside this takes about twenty seconds. From inside it takes no time at all, because inside is precisely what has been switched off.

What entered the vein was propofol, and what propofol did was find a pocket. Its target is the GABA-A receptor, the fast brake of the vertebrate brain: a pentamer, five protein subunits standing in a ring around a central pore like staves around a barrel, opening to admit chloride ions and so pushing the cell further from firing. Where two neighbouring staves meet, a cleft forms. Propofol and etomidate bind one particular cleft, the interface between a β subunit's outward face and an α subunit's inward face, written $\beta\alpha$. Neither drug opens the channel itself; each makes it easier for GABA – gamma-aminobutyric acid – to open it. The syringe adds nothing to your brain. It leans on a lever already installed.

Which lever matters. Reviewing the subunit pharmacology in the *British Journal of Anaesthesia* in 2017, Hemmings and colleagues set out a dissociation that surgery quietly depends on: the $\beta 3$ subunit carries the immobilising, hypnotic effect – the going under – while a different subunit, $\alpha 5$, carries the amnesia. Unconscious and will-not-remember – this are two effects, routed through two rooms of one very old house. The rooms themselves are not old at all.

Inhibition had to be invented, and the reason is arithmetic. A network whose only word is go has no stable state between silence and seizure: every excited cell excites its neighbours, and the endings available are exhaustion or catastrophe. Something has to be able to say no. What nature reached for is almost comically economical. Take glutamate – the go signal of the last chapter – remove one carboxyl group as carbon

dioxide, and what remains is GABA. The enzyme that does the snipping, glutamic acid decarboxylase or GAD, works with pyridoxal-5'-phosphate, the active form of vitamin B6, on a catalytic fold shared across amino-acid chemistry in all three domains of life. One chemical step separates the brain's principal yes from its principal no. The brake is machined from the accelerator.

That economy is tidy enough to invite a false inference: that GAD must therefore be a single deep inheritance. The evidence says otherwise. In *Escherichia coli*, GAD runs glutamate-dependent acid resistance — each decarboxylation consumes a proton, buying roughly two hours of survival at gastric pH, as Capitani and colleagues showed structurally in *The EMBO Journal* in 2003 — and the same system runs in *Shigella*, *Listeria* and *Lactococcus*. Then the archaea break it open. A GAD homolog in the hyperthermophile *Thermococcus kodakarensis*, locus TK1814, was knocked out and found to decarboxylate aspartate instead, feeding β -alanine and coenzyme-A synthesis, with no connection to GABA or to signalling of any kind. *Pyrococcus* makes GABA; its neighbour has aimed the identical fold elsewhere. Plants aimed it elsewhere again, into the **GABA shunt** — cytosolic GAD plus two mitochondrial enzymes routing carbon out of glutamate and back into the citric-acid cycle, doubling as a stress and pH signal. This is not one commit inherited. It is a versatile tool picked up repeatedly, for local reasons.

Plants do something stranger too. In *Nature Communications* in July 2015, Ramesh and colleagues in Matthew Gilliham's Adelaide group showed GABA regulating wheat's aluminium-activated malate transporter TaALMT1 through a short motif resembling the mammalian GABA-A binding site; mutate the motif and GABA sensitivity vanishes while ordinary transport continues. Real, and specific — but not a "plant GABA receptor." ALMT is an anion transporter with no structural kinship to the pentameric channel. GABA tunes it. GABA does not gate it.

Who makes GABA and who listens for it are separate questions, and the listening side splits again. The fast branch is the Cys-loop superfamily of pentameric ligand-gated ion channels — five subunits around a central

pore, each pinned by a signature loop stitched shut between two cysteine residues, which is all the name means – propofol's barrel. In *Genome Biology* in 2005, Tasneem, Iyer, Jakobsson and Aravind found that whole architecture, ligand-binding domain and pore together, already present in several bacteria and in the archaeal genus *Methanosarcina*, and proposed a single lateral transfer into the ancestral eukaryotic lineage followed by one duplication into a cation-selective branch (nicotinic acetylcholine, serotonin-3) and an anion-selective branch (GABA-A, glycine). Two years later Bocquet, Corringer, Changeux and colleagues gave the claim a body: GLIC, a working, proton-gated pentamer from the cyanobacterium *Gloeobacter violaceus*. The barrel is bacterial.

What animals built on it was, for a long stretch, less than the textbooks imply. Tsang, Ng, Xu and Xue reported in 2007 that invertebrates carry only one ancestral GABA-A/glycine-receptor-like gene pair. Separate GABA-A and glycine receptors as distinct proteins, the α , β and γ families, the four human clusters on chromosomes 4, 5, 15 and X – all vertebrate, built on two whole-genome duplications plus one tandem duplication. $\beta 3$ and $\alpha 5$ sit as neighbours in the chromosome-15 cluster. The dissociation the anaesthetist relies on is younger than jaws.

The slow branch runs deeper. GABA-B receptors are Family-C G-protein-coupled receptors, which conduct no ions but start an internal cascade instead – seconds, not milliseconds. Krishnan and Schiöth's group counted 33 Family-C GPCRs in the demosponge *Amphimedon queenslandica* in 2014, mGluR/GABA-B-type homologs among them, in an animal Vol. I already showed owns a postsynaptic scaffold and no neurons. In the sea anemone *Nematostella vectensis*, four GABA-B homologs carry conserved GABA- and baclofen-binding residues, and baclofen arrests the planula-to-polyp transition and blunts neurogenesis – work still a bioRxiv preprint from April 2019, its peer-review status unconfirmed. The ordering inverts the intuition: slow GABA sensing is confirmed in cnidarians and structurally present in sponges, while fast, synapse-grade, GABA-A-type inhibition has no confirmed instance anywhere below Bilateria.

Glycine's half was settled by poison and by cats. Strychnine — isolated from *Strychnos nux-vomica* seeds by Pelletier and Caventou in 1818–19, thereafter the reliable villain of detective fiction — is a competitive antagonist at the glycine receptor, and it kills by disinhibition: it excites nothing, it merely lifts glycine's brake off motor neurons, and the body convulses itself to death on its own reflexes. Glycine's day job, dominant fast inhibition in spinal cord and brainstem, was pinned down by Aprison and Werman in 1965, measuring free amino acids in cat spinal cord and finding ventral-horn glycine above both GABA and glutamate exactly where inhibitory interneurons cluster. GABA's own confirmation came a year later, when Otsuka, Iversen, Hall and Kravitz caught it leaving an identified inhibitory axon on stimulation — in a lobster whose posture Vol. I's eleventh chapter would later turn on. Glycine works the far side of the wall as well, as a required co-agonist at the excitatory NMDA receptor, though in the forebrain that seat is largely taken by D-serine, identified by Wolosker and colleagues in 2000 and three to four times more potent there. Its maker, serine racemase, is far older than the job: the gene family spans at least eight invertebrate phyla and first duplicates at the eumetazoan ancestor, after sponges branched off. Old enzyme, late wiring — GABA's story told small.

When Homology Lies

The sharpest test arrived in 2025, and it failed the intuition badly. *Nematostella*'s genome holds 42 channels of the GABA-A/glycine superfamily, and 37 of them fall in a cnidarian-specific radiation that looks, by every sequence metric available, like a set of GABA-A receptors; *Hydra* contributes ten more to the same radiation. Ojha and colleagues cloned seven testable members of the *Nematostella* set and expressed them in *Xenopus* oocytes — frog eggs, the standard host for asking a channel what it listens for.

None responded to GABA. None responded to glycine. Three responded to **glutamate**, and were renamed nvGluCl-1, -2 and -3. They are genuinely chloride-selective — substituting bulky gluconate for chloride

shifted the reversal potential exactly as a chloride channel requires — and picrotoxin, the classic GABA-A blocker, blocks them like the real thing. The glutamate response runs through a single lysine, K229; mutate it to threonine and the response is gone. The work was posted as a bioRxiv preprint in July 2025 and published in *Advanced Science* in May 2026; the ledger below grades it as peer-reviewed.

The lesson is not that the channels are fake. They are real, ancient, pentameric, chloride-conducting and inhibitory. The lesson is that ligand identity — the actual word a channel listens for — is cheap to gain and cheap to lose, and one lysine can move it. Homology-based annotation, the workhorse that assigns names to genes in every newly sequenced genome, said GABA-A receptor and was wrong about the only thing that mattered. Function must be shown, never inferred from resemblance. Ctenophores sharpen the point: their genomes, as Vol. I's ninth chapter established, carry ionotropic glutamate receptors and no Cys-loop anion-channel superfamily at all.

The same discipline handles the supplement shelf, honestly rather than cheaply. The blood-brain-barrier literature has contradicted itself since 1958: studies in 1958, 1971 and 1988 found no crossing, four more between 1980 and 2002 found minuscule crossing, and the question has never been tested directly in humans. In mice, GABA leaves the brain roughly seventeen times faster than it enters — which alone could imitate impermeability in an organ that demonstrably carries GABA transporters. What the gut itself does with the molecule is better established: Philip Strandwitz's group isolated KLE1738, a human gut bacterium that grows on GABA and on nothing else they tested, depending on *Bacteroides fragilis* to release it — a resident of the human body whose only known food is the brain's word for no. On benefit, Hepsomali, Groeger, Nishihira and Scholey's review of fourteen placebo-controlled human trials found limited evidence for stress and very limited evidence for sleep. So: we do not know whether or how oral GABA reaches a brain, and the trial evidence is thin. Not fake. Not proven. Meanwhile the receptor family anaesthetists silence outright is the same one whose autoimmune destruction — anti-GAD65 antibodies, sometimes alongside glycine-

receptor antibodies — produces the rigidity and spasm of stiff-person syndrome. This is not a mood molecule. It is a load-bearing dial, and bodies break when it slips.

The honest standing is a gap with a definite shape. Both halves of inhibition are old, and old separately: a promiscuous decarboxylase making GABA in some lineages and quite different things in others, and a bacterial barrel borrowed for purposes nobody has recovered. What no one has dated is the coincidence — the moment a lineage that happened to synthesise GABA also happened to evolve a pocket that recognised it, in the same cells, at the same time. That conjunction, not either part alone, is the commit worth finding, and the log is blank exactly where it should read. The next molecule made the opposite bargain: it committed early, spread everywhere, and settled most of itself somewhere with nothing to do with thinking at all.

Commit Ledger

| Function | First commit | Grade | What would move the date |

|---|---|---|---|

| GABA synthesis (GAD, as metabolite) | No single-origin date defensible; GABA-specific activity looks polyphyletic — *Pyrococcus* positive, *Thermococcus* repurposed to aspartate decarboxylase (comparative genomics, confirmed by knockout) | **C** | A dated GAD-specific phylogeny with ancestral-substrate reconstruction across Archaea |

| GABA shunt in plants | Undated in sources located; called "ancient and conserved" without a cited phylogeny (assertion only) | **D** | A dated GAD phylogeny across charophyte algae → bryophytes → vascular plants |

| Cys-loop channel architecture, before any ligand specificity | Present in bacteria and one archaeal genus, therefore necessarily pre-animal; no numeric date — comparative genomics plus one solved bacterial structure | **B** | Resolving single lateral transfer against multiple transfers or vertical inheritance |

| GABA-A/glycine-receptor-like gene pair (existence; ligand unconfirmed) | $\leq$ the bilaterian ancestor: crown Bilateria 595.7–688.3 Mya, Bayesian molecular clock (dos Reis et al. 2015, via Vol. I ch. 09) | **B** | Functional deorphanisation of this exact gene pair in a basal bilaterian |

| Fast ionotropic GABA-gated inhibition (true GABA-A function) | Not demonstrated below Bilateria as of 2026, and absent in *Nematostella* on functional test; lower bound unknown, upper bound $\leq$ crown Eumetazoa 625.9–746.2 Mya, Bayesian clock (via Vol. I ch. 09) | **A** for the negative result (expression + mutagenesis) · **D** for any positive date | A cloned, GABA-responsive Cys-loop channel from any early bilaterian |

| GABA-B (metabotropic) receptor existence | Functionally active in Cnidaria, structurally present in the pre-neural demosponge *Amphimedon*; genome survey plus pharmacology, no clock date | **B** overall · **C** for sponge-level binding (homology only) | A ligand-binding or functional test of the sponge Family-C homologs |

| GABA confirmed as a synaptic inhibitory transmitter | 1966 – stimulus-evoked release from an identified inhibitory axon; documentary record, not a clock estimate | **A** (1950 discovery, 1957–63 localisation, 1966 release) | Not applicable – historical record |

| Glycine confirmed as an inhibitory transmitter | 1965 – regional concentration measurement in cat spinal cord, corroborated later | **B** | Not applicable – historical record |

| D-serine as NMDA co-agonist (human-salient role) | 2000 – biochemical identification, widely replicated; the racemase family first duplicates at the eumetazoan ancestor, after the sponge split (comparative genomics) | **A** function · **C** age of the regulated enzyme | Ancestral-state reconstruction of serine-racemase regulation outside chordates |

| Inhibition itself (fast chemically gated hyperpolarisation, any ligand) | Confirmed in Cnidaria – glutamate-gated chloride channels and GABA-B effects in *Nematostella* and *Hydra*; whether it predates the cnidarian/ctenophore split is unresolved, ctenophores carrying no Cys-loop anion-

channel superfamily | **B** in Cnidaria · **D** before Cnidaria | A confirmed anion-selective Cys-loop channel, any ligand, in a ctenophore genome |

Overtuned if: a GABA-A/glycine-receptor-like gene from a basal bilaterian invertebrate is functionally cloned and shown to respond to GABA itself, pushing true ionotropic GABA-gated inhibition into or below early Bilateria rather than leaving it, as the evidence now does, effectively undated there.

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04. Serotonin: The Thermostat

A blade slips in a kitchen and opens a shallow cut across a fingertip. Within seconds platelets are piling into the gap and gluing themselves together, and every one of them carries serotonin it did not make. Platelets cannot build the molecule at all: they scavenged it from blood plasma using SERT, the serotonin transporter, and packed it into two to eight dense granules apiece using a vesicular transporter called VMAT-1. At the wound they degranulate, and the spilled serotonin lands back on their own surface receptors, 5-HT_{2A} and 5-HT₃. Aggregation amplifies. The vessel constricts. The bleeding slows, in a closed chemical loop with no nerve involved anywhere in it. The most famous brain chemical in the world has just worked a shift as a logistics contractor.

Between ninety and ninety-five percent of the serotonin in a human body — a widely repeated range rather than one traceable measurement — is made and stored in the gut, by enterochromaffin cells, fewer than one percent of the cells lining it. In 2017 Nicholas Bellono, James Bayrer, David Julius and colleagues showed in *Cell* what those cells are: chemosensors wired to sensory nerves. They register stretch, chemical irritants and bacterial byproducts, then release serotonin onto sensory nerve terminals and onto the enteric neurons Michael Gershon popularised in 1998 as the "second brain." They are not built from neural crest, the tissue supplying most neuroendocrine cell types; developmental work places them in gut endoderm. Moving a meal and closing a wound: two jobs, no synapse in either.

Vol. I dated part of this molecule's past. It followed serotonin's synthesizing enzyme, tryptophan hydroxylase, deep past animals into a prokaryotic pterin-dependent hydroxylase family; found the whole amine kit already running in a ciliate with no nervous system; and watched a lobster rise on an injection of serotonin, then watched the argument that followed. What it never dated was the receiving end. A molecule with no receptor is a chemical, not a signal, and the receptors are where this chapter's commits live.

There are fourteen of them in a human, drawn from about eighteen genes and sorted into seven families, 5-HT1 through 5-HT7. Thirteen subtypes across six families are G-protein-coupled receptors — proteins that thread the membrane seven times and pass the message inward through a G protein, slowly. One family is not. 5-HT3 is a ligand-gated ion channel from the Cys-loop superfamily: serotonin binds, a pore opens, cations pour through in milliseconds. An unrelated structural class, bolted onto the same ligand. The first 5-HT7-type receptor ever cloned came out of a fruit fly, and the mammalian version was found by homology to the fly's gene, not the reverse.

Nematodes built their own. *C. elegans* has MOD-1, described by Rajesh Ranganathan, Stephen Cannon and Robert Horvitz in *Nature* in 2000: a serotonin-gated chloride channel, about thirty percent identical to GABA and glycine receptors, ionotropic like 5-HT3 but passing anions instead of cations, and absent from vertebrates. Vol. I met the same shape in an octopus's fast dopamine receptor. The molecule is inherited once; a fast way of hearing it is invented more than once, on whatever scaffold a lineage has to hand.

One receptor carries most of the molecule's fame. LSD and psilocybin are agonists at 5-HT2A, and that single receptor anchors their effects on human perception — the pharmacology David Nichols set out in *Pharmacological Reviews* in 2016. But fame is not function, and the popular name for this molecule fails on the evidence. Deakin and Graeff's account has serotonin processing aversive events, not rewarding ones; and in 2009 Molly Crockett, Luke Clark and Trevor Robbins showed in *The Journal of Neuroscience* that acutely depleting tryptophan, serotonin's raw material, abolishes punishment-induced slowing of behaviour specifically, leaving reward-driven responding intact. A molecule whose most reproducible instruction is wait is a poor candidate for a happiness chemical. It is closer to a thermostat: it does not choose the temperature, it holds one.

So when was the thermostat installed? For the receptor family there is one number in the literature, and it is thirty years old. In 1994 Stephen

Peroutka and T. A. Howell found under twenty-five percent homology between the three major classes of 5-HT G-protein-coupled receptor and ran that divergence against a fixed clock of roughly one percent change per ten million years. Out came an age for the primordial 5-HT receptor of more than 700 to 800 million years. Set that beside this library's floor for the first animals — crown Metazoa at 649.8 to 833.5 million years, from dos Reis and colleagues' Bayesian analysis in *Current Biology* in 2015 — and the receptor date presses against the old end of that window while sitting, at its young end, older than animals are permitted to be. Either that is a real result about a pre-animal ancestor or it is an artefact: a fixed rate with no fossil calibration and no relaxed-rate model is the instrument Vol. I warned about, and nobody has redone this family the modern way. The absence is the state of the art, not a footnote.

What survives without the clock is firmer: 5-HT1-, 5-HT2- and 5-HT7-type receptors cloned directly out of invertebrates put the family in place before the protostome–deuterostome split — older than 600 million years conventionally, 642 to 761 by clock, 558 to 575 when the rocks are allowed to calibrate.

The Argument at the Bilaterian Line

The boundary below that one is live. In June 2023 Matthew Goulty, Roberto Feuda and colleagues argued in *Nature Communications* that the whole monoaminergic system — synthesis enzymes, vesicular transporters, receptors — is a bilaterian innovation, absent from cnidarians, placozoans, ctenophores and sponges. In 2026 Luis Yañez-Guerra, Gáspár Jékely and colleagues contested that in *Current Biology*. They deorphanized five G-protein-coupled receptors from the placozoan *Trichoplax adhaerens* — deorphanizing being the work of finding a catalogued receptor's natural ligand — and four of the five fall inside the bilaterian melatonin-receptor clade. All five answer to tyramine, tryptamine or phenethylamine — trace amines a placozoan builds in one enzymatic step. Serotonin bound too, but weakly — two to three orders

of magnitude worse — and placozoans lack tryptophan hydroxylase and AANAT outright, so they cannot make serotonin or melatonin at all. The honest reading is narrower than either headline. The receptor scaffold predates Bilateria; serotonin-specific signalling still looks bilaterian or later — the order Vol. I found in a sponge carrying a postsynaptic scaffold with no receptors to dock onto it. Jékely, whom Vol. I met establishing that noradrenaline-type signalling coexisted with octopamine in the last bilaterian ancestor, is making the same case again: the parts are older than the job.

Melatonin takes one line: serotonin's night-shift derivative, acetylated by AANAT — an enzyme whose activity swings roughly a hundredfold between day and night — then methylated. Vol. I fixed its origin at about 3.0 to 2.5 billion years ago, an order-of-magnitude geological inference tied to the Great Oxidation Event rather than a clock. The derivative has a date. The apparatus that reads the parent does not.

Which leaves the trap. Fluoxetine was found on 24 July 1972 by David Wong, Ray Fuller, Bryan Molloy and Klaus Schmiegell at Eli Lilly, as compound Lilly 110140, and approved as Prozac on 29 December 1987. Its mechanism is not in dispute: it blocks SERT — the transporter the platelets in the opening scene use — so less serotonin is retrieved from the synapse. That transporter's ancestry is a fold, not a function: its architecture was solved through a bacterial leucine carrier that moves no amine at all. Whether blocking SERT treats depression by correcting a serotonin deficit is a separate claim, and contested. Andrea Cipriani and colleagues found in *The Lancet* in 2018 that all twenty-one antidepressants studied beat placebo, at a modest pooled effect whose clinical meaning is argued over. Joanna Moncrieff and colleagues found in *Molecular Psychiatry* in 2022 — across serotonin and metabolite levels, receptor and transporter imaging, depletion and gene-association studies — no consistent evidence that depression is caused by lowered serotonin; the review drew a formal reply in the same journal in 2023. Efficacy and mechanism are separable claims, neither settled here, and mood biology has its own volume in this library. This one dates commits.

The dates worth trusting here are about parts, not jobs. The synthesis enzyme has a lineage and no clock; the receptor family has a clock and no calibration; the transporter has a fold and no dated cargo; and the two jobs this molecule most visibly does in a human body have no phylogenetic date at all. A thin log for the most talked-about molecule in the human head — and the thinness is the finding. The next molecule in this book does not ask an animal to hold steady; it asks it to go and get.

Commit Ledger

| Function | First commit | Grade | What would move the date |

|---|---|---|---|

| Synthesis (tryptophan hydroxylase) | Undated by clock; traced by sequence to a pre-animal pterin-dependent hydroxylase family, and absent from sponges, ctenophores and placozoans | D | A fossil-calibrated relaxed-clock tree of that hydroxylase family |

| Receptor family (5-HT GPCR radiation) | >700–800 Mya on a fixed-rate 1994 substitution clock; independently, before the protostome–deuterostome split (>600 Mya conventional; 642–761 Mya clock; 558–575 Mya fossil-calibrated) by invertebrate cloning | B | A Bayesian relaxed-clock, multi-calibration reanalysis of the family — not yet attempted |

| Fast (ionotropic) reception | Undated; a Cys-loop scaffold recruited at least twice — cation channel in vertebrates, anion channel in nematodes, neither in the other | D | A calibrated Cys-loop phylogeny placing each serotonin-gated branch |

| Transporter (SERT): fold vs. cargo | Fold pre-bilaterian, traced into bacteria by crystallography of LeuT; amine-specific transport undated | C | A bacterial LeuT-fold protein shown to move an amine naturally |

| Serotonin-specific neural signalling | Contested at the Bilateria boundary: a bilaterian innovation on 2023 comparative genomics versus a pre-bilaterian trace-amine-tuned scaffold on 2026 deorphanization | C |

Serotonin-specific, not trace-amine-general, signalling shown functionally in a non-bilaterian |

| Gut (enterochromaffin) role | Undated phylogenetically; developmental evidence only – endodermal origin, not neural crest | D | A cross-species clock or fossil correlate for enterochromaffin-cell origin |

| Platelet and clotting role | Cannot predate the SERT and VMAT-1 machinery it borrows; platelets synthesize none of it, and SERT's own date is unfixed | D | A date for SERT's split from the sister monoamine transporters |

| Mood-relevant human role | **Undatable as stated** – mood is not a discrete phylogenetic event, and the present-day mechanism in living humans is unresolved | D | Nothing: a mechanism question, not a timing question |

Overtured if: a living non-bilaterian runs serotonin-specific signalling functionally – own synthesis, own selective receptor, a behaviour that changes when either is blocked – rather than a promiscuous trace-amine receptor serotonin happens to fit.

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05. Dopamine: The Wanting Machine

In the spring of 1969, on the wards of Beth Abraham Hospital in the Bronx, roughly eighty people who had been unable to begin a movement for decades were started on L-DOPA. They were survivors of encephalitis lethargica, the sleeping-sickness epidemic of the 1920s, left in post-encephalitic parkinsonism — awake and aware inside bodies that would not start. Their physician was Oliver Sacks. Over the following weeks the wards came back: patients moved, spoke, and were recognisably themselves again. Then the effect frayed into tolerance and dyskinesia — movement arriving unbidden and refusing to stop — and most of them settled back toward stillness. Sacks published the account in 1973 as *Awakenings*.

That ward is the most complete demonstration this book has of what a signalling molecule does for a human being, and it ran backwards. Nobody there was shown dopamine working; they were shown its absence, then its brief return, then its absence again. This is the molecule's peculiar biography: we know it best by subtraction.

The subtraction was a laboratory method before it was a clinical one. In 1957 Arvid Carlsson, with Lindqvist and Magnusson, gave rabbits reserpine — a drug that strips the brain of its dopamine — and watched the animals go rigid and immobile. Injecting L-DOPA brought them back. Injecting 5-hydroxytryptophan, the precursor of serotonin, did not. That second injection is the whole result: it separates dopamine from the molecules beside it on the same chemical shelf and shows it driving movement in its own right, not merely as a way-station toward something else. Published in *Nature*, the finding carried a Nobel Prize in 2000 and is the direct ancestor of the bottle that reached Sacks's ward twelve years later.

What Sacks handed his patients was not dopamine but the step before it. The line is two enzymes long: the amino acid tyrosine is hydroxylated by tyrosine hydroxylase into L-DOPA, and L-DOPA has a carboxyl group cut

away by DOPA decarboxylase to become dopamine. Vol. I traced the first of those enzymes into a pterin-dependent hydroxylase family older than animals, and watched a water flea run both in the wall of its gut. Plants build dopamine by their own convergent routes; a ciliate with no nervous system at all makes it and secretes it. None of this is vertebrate equipment, and it is why the treatment could work at all: Sacks supplied the intermediate and let each patient's own decarboxylase perform the final step. He was not delivering a signal. He was restocking a shelf.

Making a molecule and hearing one are different achievements, and they carry different dates. Vol. I's chapter on the vertebrate bargain found the full mammalian-style D1/D2 architecture, direct and indirect pathways included, already complete in a lamprey, whose lineage parted from ours around 560 million years ago. But the receptor inventory is a hundred million years younger. A whole-genome duplication near the root of Vertebrata, placed in the Ordovician around 450 million years ago by comparative genomics, took a single ancestral D1/D2 pair and made four D1-like receptors and several D2-like ones. Architecture at 560. Count at 450. One word — receptor — two separate commits underneath it.

One lead belongs in the margin rather than the ledger. A claim surfaced during this book's sourcing, in search summaries only, that D1-like and D2-like receptors are not sister lineages at all but independent arrivals at the same ligand — convergence rather than duplication. Two attempts to reach the primary source were blocked, and this book's rule is that an unverified claim is recorded as a question or not at all. This one stays a question. Note only what it would cost if it held: not the 450-million-year row, which counts duplications, but the assumption beneath it that "the ancestral D1/D2 pair" is one object worth counting duplications of.

Below the vertebrates the receptor's floor gets harder to find, and the sharpest evidence is negative. Ctenophores — the comb jellies of Vol. I's chapter on the first animals — run a working nervous system with no dopamine signalling in it whatsoever, which puts a ceiling on how deep the receptor can reach. A 2023 phylogenetic reconciliation by Goulty, Botton-Amiot, Rosato, Sprecher and Feuda presses the same direction in

its title, calling the monoaminergic system as a whole a bilaterian innovation — a claim about the family, not a direct test of the dopamine receptor. Nor is the receptor's shape fixed above that line: Vol. I describes an octopus dopamine receptor, DopC1, that is ionotropic — a channel opening directly and fast — where every vertebrate dopamine receptor is a slow G-protein-coupled one. It stands as a 2025 preprint, not yet peer-reviewed, a caveat worth carrying alongside it. Meanwhile the synthesis line was already running, in gut walls and in single cells with no neurons to run it for.

Keeping the Rows Apart

"How old is dopamine" is not one question, and the reason this book keeps a ledger instead of a timeline is that it has at least four defensible answers separated by more than a hundred million years. The first is the synthesis capability — could a cell make the molecule? — and it goes back at least 600 million years, to at or before the last common ancestor of protostomes and deuterostomes. Vol. I is careful with that anchor and this book will be too: conventionally cited above 600 million years, it arrives at 642 to 761 million by molecular clock and around 558 to 575 by fossil calibration, and the distance between rocks and clocks is unresolved rather than split. The second answer is receptor existence — was there anything built to hear it? The third is circuit — was it wired into a loop that picks one action and suppresses the rest? The fourth is the job a human would actually name: wanting.

The fourth is the weakest row here, and the method is to say so rather than let the first three carry it across. Vol. I told how monkey dopamine neurons were caught encoding a reward-prediction error, and how rats made to want sucrose more did not come to like it any better. Both are findings about a computation running on the molecule, not about the molecule. Nobody has recorded that burst-and-dip signature in a lamprey or a hagfish — the two animals whose ancient basal ganglia would settle whether the computation is as old as the circuit or a much later tenant of it. The molecule has a date. The computation has none. An

undated row is not an empty row; it is a row with an experiment still owed.

Outside the laboratory this bookkeeping stops being bookkeeping and becomes a defence. The popular account collapses all four rows into a single quantity: a "hit" that arrives as pleasure, drains with use, and can be restocked by abstinence — the dopamine detox. Each clause is wrong at a different row. The pleasure claim is the one Vol. I's rats already put down. The draining claim has no reservoir to drain: dopamine, as The Scientist's 2024 debunking of the detox trend puts it, "is essentially never acting in isolation" — other neuron populations gate the dopaminergic ones, and endogenous opioids, orexin and serotonin carry much of what the slang credits to dopamine alone. There is nothing for a fast to act on. The phone in your hand is misdescribed by the slang too: the firing comes before the notification resolves, keyed to the uncertainty rather than the reward — a prediction system doing its job, not a dose landing. And if you want to see dopamine depletion in a human being, it is not someone scrolling with dulled enthusiasm. It is the ward at Beth Abraham in the weeks before the L-DOPA.

The honest standing is uneven, and the unevenness is the finding. The synthesis rows are solid and very old; the circuit row is solid and younger; the receptor rows are solid and split across two dates the popular question flattens into one; and the row naming what a human actually feels is the weakest line here. That asymmetry is not a gap in the evidence, it is the evidence: this molecule was doing chemistry for hundreds of millions of years before anything used it to want. Dopamine also does not end at dopamine. It sits mid-line — an intermediate that acquired a receptor and got promoted to a signal — and the cheapest way evolution has found to make a new message is to bolt one more enzyme onto a line already running. One enzyme downstream of wanting is noradrenaline, and the body uses it to sound an alarm.

Commit Ledger

| Function | First commit | Grade | What would move the date |

|---|---|---|---|

| Synthesis capability (tyrosine → L-DOPA → dopamine; TH then DDC) |
≥600 Mya, at or before the protostome–deuterostome LCA; method-split
anchor — 642–761 Mya molecular clock vs. ~558–575 Mya fossil calibration
| B | Confirmed non-neural dopamine use in a cnidarian running
homologous enzymes |

| Receptor existence (something built to bind dopamine) | At or below the
bilaterian LCA, inside the same 600–760 Mya window; ceiling set by
ctenophore genomes carrying none — comparative genomics | B | A
confirmed dopamine-receptor ortholog in a ctenophore or a cnidarian |

| Circuit architecture (D1/D2 direct-indirect basal ganglia loop) | At or
before ~560 Mya, late Ediacaran — dated by the cyclostome/
gnathostome split; comparative neuroanatomy in lamprey | B | Revision of
the cyclostome split date; no earlier direct test of the circuit exists |

| Receptor inventory expansion (one D1/D2 pair → 4 D1-like + several D2-
like) | At or near 450 Mya, Ordovician — vertebrate whole-genome-
duplication timing; comparative genomics | B | Revision of vertebrate 2R-
WGD timing generally, not of anything dopamine-specific |

| Ionotropic (fast, channel-type) dopamine reception | Undated;
cephalopod-restricted so far — structural inference from octopus DopC1,
2025 preprint, not peer-reviewed | C | Peer review of the DopC1 preprint,
or a second ionotropic dopamine receptor in another lineage |

| Reward-prediction-error computation — the human-salient "wanting"
role | Undated. The molecule is ≥600 My old; the computation has no
confirmed pre-teleost date located for this book | D | Canonical burst/dip
RPE signatures recorded in lamprey or hagfish electrophysiology |

Overtured if: a functional dopamine receptor is confirmed in a
ctenophore genome — the one lineage that runs a nervous system with
none of this chemistry inside it — which would break the bilaterian
ceiling drawn here and reopen every receptor row beneath 600 million
years.

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06. Noradrenaline and Octopamine: The Alarm Twins

At the Maudsley Hospital in south London, in the early 1960s, patients taking a new class of antidepressant kept returning with the same account: a headache arriving within an hour of a meal, felt at the back of the skull as something being forced open, and behind it a blood pressure high enough to rupture a vessel in the brain. Blackwell collected twelve such cases — eleven of the patients on tranylcypromine, one on phenelzine, all of them monoamine-oxidase inhibitors, drugs that work by disabling the enzyme that strips small amine messengers out of circulation. His report reached *The Lancet* in October 1963 under a title that named the drug class but not the trigger. The trigger was cheese.

The chemistry underneath is exact and slightly absurd. Ripening bacteria decarboxylate the amino acid tyrosine — snip a carbon dioxide off one end — leaving tyramine, a small amine otherwise of almost no consequence to a human being. In an unmedicated person it barely exists as a signal: monoamine oxidase in the gut wall and the liver destroys nearly all of it before it reaches the bloodstream. Block that enzyme and the tyramine arrives intact, is carried into sympathetic nerve endings by the very transporter that normally recycles noradrenaline, and displaces the stored noradrenaline there in one mass release. The result is every effect of a maximal fright — vessels clamped, pressure spiking, heart driven hard — delivered at once and for no reason at all.

What makes this more than a pharmacological footnote is which molecule did it. In a honeybee or a fruit fly, tyramine is a signalling molecule in its own right, with dedicated receptors and behavioural jobs; in a human it is a trace amine — a category defined by scarcity, cleared so fast it was long treated as metabolic litter. The cheese reaction is the one everyday place where an invertebrate's messenger turns acutely, dangerously human.

That crossover is not a coincidence, and the reason is that the two branches were never separate inventions. One assembly line runs from a common amino acid: tyrosine, hydroxylated by tyrosine hydroxylase into L-DOPA, decarboxylated by DOPA decarboxylase into dopamine — the two steps the previous chapter follows to their own conclusions. Add one more enzyme, dopamine β -hydroxylase, and dopamine becomes noradrenaline. Add one after that, phenylethanolamine N-methyltransferase, and noradrenaline becomes adrenaline. Each addition is a separate commit with its own date, and the dates are not close together.

The parallel branch does the same two operations in the opposite order. Decarboxylate tyrosine first and you get tyramine — the cheese molecule. Hydroxylate that, and you get octopamine, the amine that runs much of an insect's arousal and energy mobilisation. Vol. I's chapter on rank showed what octopamine does to a lobster's posture with nothing but a syringe; what it did not do was date the machinery. The order of operations is the only real difference between the branches, and for most of the twentieth century that difference was taken to mean two enzyme families, one vertebrate and one invertebrate, descended from an ancient split.

That reading is now wrong, and the correction is unusually clean. In 2023, Matthew Goulty, Gaëlle Botton-Amiot, Ezio Rosato, Simon Sprecher and Roberto Feuda published a formal phylogenetic reconciliation of the monoaminergic gene families in *Nature Communications*, and found that dopamine β -hydroxylase and tyramine β -hydroxylase are not two clades at all. The fruit fly's TBH gene is a one-to-one ortholog of the human DBH gene — the same gene, inherited from the same ancestral copy, at strong branch support. In the authors' own summary, the name distinction is purely semantic. One enzyme lineage, continuously present since close to the split between cnidarians and bilaterians, performs whichever final hydroxylation the local chemistry offers it: dopamine to noradrenaline where dopamine is the abundant substrate, tyramine to octopamine where the decarboxylation ran first. The same study places the lineage's sister paralog, MOXD — still functionally uncharacterised in

any species — at the last common ancestor of Cnidaria and Bilateria, which puts the family's root deeper than crown Bilateria even though the surviving DBH and TBH genes themselves are almost entirely bilaterian.

So the naming was the accident. Two labels, coined in two research communities that rarely read each other, made the invertebrate version sound like a lesser, earlier draft of the vertebrate one. It was never a draft. It was the same gene, handed a different substrate.

The receptors run on a separate clock from the enzymes, and they are where the twins story actually lives. Vol. I established, through Gáspár Jékely's work, that noradrenaline-type signalling coexisted with octopamine and tyramine in the last bilaterian common ancestor rather than being replaced by them. Unpacked past that summary, the 2017 reconstruction by Thomas Bauknecht and Jékely in *BMC Biology* resolves not one shared inheritance but a whole panel of them — adrenergic $\alpha 1$, $\alpha 2$ and β ; octopamine α and β ; tyramine type-1 and type-2 — every family traceable to the same bilaterian node, and every family carrying its own separate history of loss. Adrenergic receptors are gone from most ecdysozoans, including most insects, yet kept in the water flea *Daphnia pulex* and in a priapulid worm; octopamine and tyramine receptors are gone from most deuterostomes, yet kept in the hemichordate *Saccoglossus kowalevskii*; the adrenergic- β family turns up only in chordates and one xenacoelomorph, the marine worm *Meara stichopi*. There is no relay anywhere in the tree — no baton pass from an old system to a new one, only lineages independently dropping whichever half of an ancestral pair they had stopped using.

Dating that node means leaning on the anchor Vol. I already handled honestly: the protostome–deuterostome split, conventionally placed beyond 600 million years ago, given as 642–761 Mya by molecular clock and roughly 558–575 Mya when calibrated to fossils. Rocks and clocks disagree, and this book cites the disagreement rather than a single number.

Why two systems survived six hundred million years apiece, rather than one absorbing the other, is where the book's three standing hypotheses

— frozen accident, active selection, chemical constraint — pull against each other. Chemical constraint has the strongest showing, and its clearest statement is old: R. A. Jensen's 1976 argument in the *Annual Review of Microbiology* that new functions arise mostly by recruiting existing enzymes to nearby substrates rather than by inventing machinery. But selection has real evidence on the octopamine side. Herman Lehman, David Schulz, Andrew Barron, Gene Robinson and colleagues showed in 2006 that honeybee foraging onset tracks brain octopamine and TBH transcript levels as workers shift from hive duties to flight; Paul Stevenson, Varya Dyakonova, Jan Rillich and Klaus Schildberger showed in 2005 that cricket aggression escalates under octopamine agonists and collapses under antagonists, with serotonin pulling the opposite way — the same opposed-amine architecture Vol. I found in the lobster, in an animal built on a different nervous-system plan. An insect with no discrete endocrine gland needs one fast, cheap, whole-body mobilisation signal; vertebrates, once glands were available, ran the same job down two channels — a fast neural one through the sympathetic chain, a slow hormonal one through chromaffin tissue. Octopamine was not retained by inertia.

The younger twin

Adrenaline is the newcomer, and the gap is larger than most readers expect. PNMT — the enzyme that methylates noradrenaline into adrenaline — is vertebrate-only, and the 2023 reconciliation dates its origin, through a duplication it shares with two other vertebrate-exclusive methyltransferases, to the ancestor of jawed vertebrates specifically rather than vertebrates broadly. Crown Gnathostomata runs to roughly 450 million years by molecular clock, with a hard fossil floor supplied in 2022 when You-an Zhu, Per Ahlberg, Min Zhu and colleagues described complete jawed fishes from the early Silurian of China in *Nature*, at about 439 million years. Lampreys and hagfish, which left the jawed lineage around 560 Mya, carry no PNMT ortholog in this analysis. What they do carry is the gland-side hardware: Jacques Paiement and Donald McMillan described extracardiac chromaffin cells in larval

lampreys in 1975 — neural-crest-derived, noradrenaline-making, structurally the tissue a mammalian adrenal medulla is built from. The organ was there without the last enzyme. If the gnathostome-only result holds, true adrenaline is about a hundred million years younger than the noradrenaline it is made from.

The wiring is older still, and that is the part a fifty-year textbook line got wrong. Following Botar in 1974 and Burnstock in 1969, the sympathetic chain — the paired ganglia running alongside the spine that carry the fight-or-flight command — was held to be an innovation of jawed vertebrates, absent from cyclostomes. In 2024, Brittany Edens, Jan Stundl, Hugo Urrutia and Marianne Bronner reported in *Nature* that sea lamprey embryos do build trunk sympathetic neurons: sympathoblasts near the dorsal aorta, traced to trunk neural crest by dye lineage labelling, expressing both tyrosine hydroxylase and DBH, and running a transcriptional program homologous to the gnathostome one. A rudimentary sympathetic nervous system therefore dates to the base of Vertebrata, around 560 million years ago — more than a hundred million years before the molecule that now amplifies it existed. The alarm circuit was built first, and adrenaline was added to a system already running.

Which leaves the question the cheese opened. If humans keep no octopamine system, why does tyramine do anything to us at all? Because vertebrates built their own receptors for these molecules, separately. Seong-il Eyun, Hideaki Moriyama, Federico Hoffmann and Etsuko Moriyama surveyed 493 trace amine-associated receptor genes across 26 vertebrate genomes in 2016 and found the family absent from every non-vertebrate genome sampled, with the earliest recognisable member appearing in lamprey — a family roughly as old as vertebrates themselves. TAAR1, its one confirmed non-olfactory member, binds tyramine and octopamine directly. It is tempting to call it the octopamine receptor humans kept, and it is not one: as Lothar Lindemann and Marius Hoener set out in 2005, the vertebrate TAARs bear no phylogenetic relation to invertebrate octopamine and tyramine receptors. Two unrelated receptor families converged on the same small ligands. The cheese finds a lock in us that our ancestors cut independently.

Two of these dates are solid and one is fragile. The bilaterian node for the receptor families rests on a reconstruction nobody has contradicted in nine years, and the base-of-Vertebrata date for the sympathetic chain now rests on direct embryological evidence rather than on an absence of it. The gnathostome-only date for adrenaline rests on one gene family in one analysis, and a jawless-vertebrate genome carrying a PNMT-like sequence would collapse the gap tomorrow. What survives either way is the shape of the thing: an assembly line extended one enzyme at a time across six hundred million years, two branches that never competed and never merged, and alarm wiring laid down long before the molecule that now screams down it. A dose of that molecule sits in every auto-injector carried by someone whose immune system overreacts to shellfish – and the messenger doing the overreacting is another old amine holding down two unrelated jobs, met next in allergy season.

Commit Ledger

Function	First commit	Grade	What would move the date
Noradrenaline synthesis capability (DBH/TBH – one gene lineage, two substrates)	Family root at the Cnidaria-Bilateria ancestor; surviving genes effectively bilaterian – ≥600 Mya (molecular clock 642–761 Mya; fossil-calibrated ~558–575 Mya), phylogenetic reconciliation	B	A second, independent β-hydroxylase duplication, or an unambiguous cnidarian DBH/TBH gene, breaking the one-gene-two-jobs result
Adrenaline synthesis capability (PNMT)	~450 Mya, ancestor of crown Gnathostomata – molecular clock, with a fossil floor of ~439 Mya (early Silurian complete jawed fishes)	C	A PNMT ortholog, or a convergent adrenaline-synthesising route, in lamprey or hagfish genome data
Adrenergic receptor families (α1, α2 solid; β chordates plus one xenacoelomorph)	≥600 Mya, bilaterian last common ancestor for α1/α2 – comparative genomics; β's node unresolved, chordate-specific or basal		

| B | For β , settling whether xenacoelomorphs sit outside all other Bilateria; for $\alpha 1/\alpha 2$, α -type receptors recovered in a cnidarian |

| Octopamine (α , β) and tyramine (type-1, type-2) receptor families | ≥ 600 Mya, bilaterian last common ancestor – same reconstruction, same node as the adrenergic families | B | A confirmed octopamine receptor in a deuterostome outside *Saccoglossus kowalevskii* |

| Sympathetic nervous system (trunk chain ganglia) | ~ 560 Mya, base of Vertebrata – neural-crest lineage labelling and comparative expression in sea lamprey; revised up from "jawed vertebrates only" | B | Confirmed homologous sympathoblasts in hagfish, currently untested |

| Chromaffin tissue (proto-adrenal medulla), noradrenaline-only | Present in larval lamprey, so ≥ 560 Mya on the cyclostome-split calibration – comparative histology | B | A positive PNMT-activity assay in lamprey chromaffin tissue |

| Trace amine-associated receptors (incl. TAAR1) | ~ 560 Mya, base of Vertebrata – earliest member in lamprey; 493 genes across 26 genomes, absent from every non-vertebrate genome sampled | C | Denser cyclostome-stem genomic sampling, currently thin |

| Locus coeruleus as a discrete noradrenergic nucleus | ≥ 560 Mya inferred from its presence across surveyed vertebrates on the cyclostome-split calibration – pooled cross-species electrophysiology, preprint; no phylogenetic dating attempted | C | Peer-reviewed publication of the pooled dataset; a cyclostome recording would test the inference directly |

Overtaken if: a PNMT ortholog, or a convergent adrenaline-synthesising route, is confirmed in a lamprey or hagfish – erasing the roughly hundred-million-year gap this chapter draws between the noradrenaline commit and the adrenaline one.

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07. Histamine: The Double Agent

The bite is already swallowed when the body starts arguing with it. A shellfish protein finds IgE antibodies docked on mast cells — granule-stuffed immune cells stationed in skin, gut lining and airway wall. Two antibodies bind the same protein, their receptors are dragged together, and within five to thirty minutes the granules empty: histamine, already made and stored, with tryptase and carboxypeptidase A behind it. Histamine at H1 receptors opens the seams between the cells lining blood vessels. Plasma leaves the circulation, pressure falls, airway muscle tightens, the gut cramps. One molecule does most of the visible damage.

The pen that stops it never touches histamine. Epinephrine — adrenaline, the previous chapter's molecule — works three fronts at once: alpha-1 constriction to reverse the collapse in pressure, beta-1 to raise heart rate and force, beta-2 to open the airway and tell the mast cell to stop releasing. The rescue drug for a histamine emergency is the last chapter's alarm molecule, and the two meet inside one quarter of an hour, pulling opposite ways.

Histamine holds a second job with no connection to immunity. Its only neuronal source in the brain is the tuberomammillary nucleus of the posterior hypothalamus. Those neurons fire around five times a second shortly after waking, fall silent through non-REM and REM sleep, and project widely enough to lift arousal, cognition and movement network-wide; most co-release GABA, so the signal carries its own brake. Takeo Yoshikawa and colleagues set the circuit out in 2021. One molecule, two unrelated employers.

Which is why an afternoon goes soft after an allergy tablet. First-generation antihistamines — diphenhydramine, chlorpheniramine, the original mepyramine — are small, fat-soluble, and not substrates for P-glycoprotein, the pump guarding the blood-brain barrier. They cross freely and occupy over seventy percent of the brain's H1 receptors, measured by positron-emission tomography with radiolabelled doxepin.

Loratadine, fexofenadine and cetirizine are larger, more charged, largely kept out. Non-drowsy is pharmacokinetics, not mechanism.

The human arc runs through two Nobel Prizes. In 1910 Henry Dale and Patrick Laidlaw published thirty-six pages in the *Journal of Physiology* on β -iminazolyethylamine: it stimulated smooth muscle, dropped blood pressure, and produced a picture they noted resembled anaphylactic shock — before anyone had established that a body makes it. Daniel Bovet, in Ernest Fourneau's laboratory at the Pasteur Institute, built the compounds that blocked it and took the 1957 prize alone, for "synthetic compounds that inhibit the action of certain body substances."

James Black's insight was a negative result: every antihistamine then in use left gastric acid secretion untouched, so a second receptor had to exist. Black, with Duncan, Durant, Ganellin and Parsons, defined it in *Nature* in April 1972 with a purpose-built blocker, burimamide; cimetidine followed, a stomach drug not an allergy drug, and Black shared the 1988 prize. The classes are still confused, so: H1 blockers treat allergy, H2 blockers treat acid, and the drowsy split cuts across neither.

Then there are the flies that see with it. In adult *Drosophila* the photoreceptors R1–R6 and R8 release histamine, and essentially only histamine, into the lamina beneath the compound eye — P. Vijay Sarthy's 1991 case, with immunostaining replicated in the blowfly *Calliphora erythrocephala* and the housefly *Musca domestica*. Vision here runs on recycling: glia take it up, conjugate it to beta-alanine as carbinine, and the transporter CarT hauls it back; delete CarT and vision fails. Fly neurogenetics holds the receiving end is an ionotropic histamine-gated chloride channel, HisCl1 or ort, unrelated to H1–H4 — a reading not re-verified here, carried as probable.

The molecule also arrives at dinner. Dark-muscle fish — tuna, mackerel, mahi-mahi, amberjack — carry high free histidine in the flesh. Chill them too slowly and gram-negative bacteria already in gut and gills decarboxylate it into histamine; above about 26°C toxic levels build within roughly twelve hours, and the histamine survives cooking, freezing and canning. Flushing, headache, palpitations and cramps follow

within the hour. John Morrow and colleagues named histamine the causative toxin of scombroid poisoning in 1991 — and that enzyme belongs to the patchy, laterally-shared bacterial toolkit Vol. I already traced.

Scombroid should be kept clear of what it gets confused with. Diamine oxidase does degrade dietary histamine, and that biology is real; but histamine intolerance as practised has no validated biomarker, diagnosis resting on elimination and reintroduction, with enzyme-supplement trials few and small. Grade it C, contested. The poisoning is measured; the intolerance is inferred.

The Control Group

This book tests three explanations for why a signalling molecule is still here: a frozen ancient commit inherited vertically from one origin; repeated reinvention, the same solution found independently more than once; and lateral spread, genes moving sideways between unrelated lineages. Histamine fails the first and demonstrates the other two. That is not a gap in the chapter. It is the chapter's job.

Start with synthesis. Histidine decarboxylase is not one enzyme quietly inherited: two structurally unrelated families reached the same trick separately, and in bacteria it carries an active transfer signature, as Daniel Wüthrich and colleagues showed for the *Lactobacillus parabuchneri* cluster in 2017. Plants accumulate histamine under stress but have no pathway of their own — the candidate plant enzyme proved a serine decarboxylase. Beneath all of it, the pathway building histidine was complete before LUCA.

The receptors complicate this from the opposite direction. Isha Ravhe, Arunkumar Krishnan and Narayanan Manoj reconstructed the family in 2020 with full-genome phylogenetics plus gene-structure and synteny checks. H2 is the deepest branch, sharing a progenitor with the alpha-adrenoreceptor in a prebilaterian ancestor — older than the protostome-deuterostome split this book leans on as its invertebrate floor. H1 shares

one with the muscarinic acetylcholine receptor in a bilaterian ancestor. H3A, H3B and H4 descend from a single H3-like progenitor through the whole-genome duplications at the vertebrate root — the bonding peptides' mechanism next chapter, not their date. Then the twist: the ability to bind histamine was acquired convergently across H1, H2 and H3/H4. The scaffolds are ancient. The job is new, three times over.

The same paper corrects a folk story. H4 is popularly the young immune specialist, and its discovery is genuinely recent — cloned in 2000 from genome-database mining. But the phylogeny finds it broadly present across vertebrates, not restricted to mammals. A later narrowing toward mast cells and eosinophils remains possible; the tree does not resolve it. Recent to us is not recent in the genome.

The immune side is older than the immune system now using it. Guang William Wong, Richard Stevens and colleagues placed mast-cell-type cells in the urochordates in 2014, before adaptive immunity existed, and Mauro Cavalcante's Rio de Janeiro group found heparin inside basophil-like circulating cells of the ascidian *Styela plicata*. The freshest evidence is Francesca Brunelli, Federico Dalle Palle and Francesca Cima's 2025 study of *Botrylloides leachii*: granular cells holding heparin and histamine in one granule, carrying mast-cell hydrolases vertebrate basophils lack, degranulating on bacterial spores, migrating into the gut epithelium under challenge. They also label with an anti-c-kit antibody — which the authors say plainly is cross-reactivity, not gene-level orthology, naming molecular confirmation as the next test. Tunicates appear in the Cambrian, roughly 518 to 500 million years ago; true vertebrate mast cells sit at the last common ancestor with hagfish, lamprey and sharks, roughly 450 to 500 million years ago.

Put it together. Histamine turns up in bacteria, in stressed plants, in a ciliate with no nervous system, in a fly's eye and in a human hypothalamus — and none of that is evidence of one deep inheritance. Presence is not descent. Histamine is what a not-frozen molecule looks like, and without it the frozen cast would be an impression rather than a measurement.

The standing is uneven and should be read that way. The precursor pathway's antiquity is as solid as deep-time inference gets; the H1 and H2 scaffolds rest on one careful study nobody has replicated; the tunicate granules rest on antibodies and micrographs awaiting a gene sequence; and the circuit that ruins an afternoon has no phylogenetic date at all. What comes next is the opposite kind of molecule: not a broadcast alarm dumped into the blood by the thousand, but nine amino acids, addressed from one body to one other.

Commit Ledger

| Function | First commit | Grade | What would move the date |

|---|---|---|---|

| Histidine biosynthesis (precursor) | Pre-LUCA, ~3.5–4 Gya — comparative genomics of the his genes | A | A redating of LUCA; a domain building histidine another way |

| Histamine synthesis (histidine decarboxylase) | No single commit — two unrelated enzyme families; an active lateral-transfer signature in bacteria | C | A basal, non-transfer-signature HDC in an early-branching lineage |

| Receptor scaffolds (H1, H2) | H2 prebilaterian, older than the protostome–deuterostome split (fossil-calibrated ~558–575 Mya; clock 642–761 Mya); H1 bilaterian — phylogenomics with synteny | B | Replication in amphioxus and lophotrochozoan genomes |

| Receptor scaffolds (H3A, H3B, H4) | One H3-like progenitor split by whole-genome duplications at the vertebrate root; placed by synteny, no interval given | C | A calibrated date for those duplications; H4 absent in lamprey or hagfish |

| Histamine-binding by a receptor | No single node — convergent in at least three lineages; structural and phylogenetic inference | C | A shared ancestral binding motif across H1, H2 and H3/H4 |

| Immune granule kit (heparin, histamine, hydrolases) | Tunicate granular cells; Tunicata from ~518–500 Mya, Cambrian fossil calibration —

immunohistochemistry and TEM | C | Gene-level c-kit or FcεRI orthology in tunicates – the source's own next test |

| "True" vertebrate mast cell | ~450–500 Mya, at the last common ancestor with hagfish, lamprey and sharks – comparative histology | B | Mast-cell hydrolase genes found, or missing, in a jawless-vertebrate genome |

| Wake-promoting neural role (TMN) | Undated – mechanism established in mammals only, no comparative phylogeny published | D | Any dated study of the arousal circuit outside mammals |

| Insect photoreceptor transmitter role | Confirmed across Diptera; no divergence-time estimate published – D on date, B on function | D | A dated survey of histamine-gated chloride channels across insect orders |

Overtured if: a shared ancestral histamine-binding motif is recovered across the H1, H2 and H3/H4 lineages, collapsing three convergent recruitments into one inherited origin and moving histamine into this book's frozen cast.

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08. Oxytocin and Vasopressin: The Bonding Fork

A labor ward, late in a long night. A bag of clear fluid hangs beside the bed, and a pump counts it down a line taped to the back of a woman's hand. In it is a chain of nine amino acids called oxytocin, built in a factory rather than a brain and sold as Pitocin. It docks onto OXTR, the oxytocin receptor studding every smooth-muscle cell of the uterine wall, which answers by releasing calcium inside the cell — and calcium is what makes muscle shorten. Half a century on, they are among the most widely administered molecules a human ever meets.

The body runs the same circuit as a loop feeding on its own output. Pressure from the descending fetus against the cervix signals the hypothalamus, which releases oxytocin from the posterior pituitary, which contracts the uterus, which drives the fetus harder against the cervix. Physiologists call it the Ferguson reflex — one of the few positive-feedback loops in a body whose control circuits almost all exist to hold a variable steady. A second loop follows birth: suckling triggers oxytocin, which squeezes the myoepithelial cells around the milk glands, and milk lets down half a minute later. Both stop only when what started them is gone.

The bag exists because of a specific chain of results. In 1906, in a paper mostly about ergot, Henry Dale reported that posterior-pituitary extract contracted a pregnant cat's uterus, and named the effect oxytocic, from the Greek for swift birth. The crude extract reached obstetric use within five years; in 1928 Kamm and colleagues at Parke, Davis split it into two active substances of unknown structure. Then in 1953 Vincent du Vigneaud, at Cornell, determined the nine-residue sequence, built the molecule from scratch, and showed it behaved exactly like the natural one. His 1955 Nobel Prize cites "the first synthesis of a polypeptide hormone." A hormone had been reduced to a sequence and a shape.

Kamm's two substances are this chapter's molecules: oxytocin and vasopressin, nine residues each, differing at two positions. In the human genome they sit almost on top of each other — OXT and AVP, roughly eleven kilobases apart on chromosome 20, transcribed toward each other from opposite strands, tail-to-tail. It is an irresistible picture: the duplication still legible, two files side by side because one was copied from the other.

The picture is backwards. Gwee, Tay, Brenner and Venkatesh sequenced the nonapeptide loci in elephant shark and Japanese lamprey and compared them with the arrangements known elsewhere. In elephant shark, coelacanth, *Xenopus*, chicken and opossum the two genes are linked tail-to-**head**, and that, on their argument, is ancestral. The tail-to-tail layout in humans and rodents is a later, lineage-specific inversion of the oxytocin gene alone. What gets pointed to as the fork made visible is a mammalian rearrangement of a fork older than the picture showing it.

The same paper settles the larger question with its best outgroups. Japanese lamprey, a living jawless vertebrate, and amphioxus, a cephalochordate more basal still, each carry exactly **one** nonapeptide gene, and both genomes were searched for a second. In each, that gene is vasopressin-family, not oxytocin-family. Amphioxus's copy, named [Ile4]vasotocin, is a 167-residue prohormone with the vertebrate four-part architecture — signal peptide, nonapeptide, neurophysin, copeptin — differing from vertebrate vasotocin at one residue. Elephant shark, the earliest-diverging jawed lineage sampled, already carries both. Vasopressin is the original file; oxytocin is the copy.

That fixes where the fork happened without dating it. Gwee's team published no clock estimate, and the honest bracket comes from two splits Vol. I already dated: the fork is younger than the jawless/jawed divergence around 560 million years ago and older than the cartilaginous/bony fish split around 450, since elephant shark already carries both. This is not the event Vol. I dated. There the six-gene family of receptors was traced to two rounds of whole-genome duplication in a 600-to-450-million-year window — Ocampo Daza, Bergqvist and

Larhammar arguing for two rounds from fourfold synteny, Theofanopoulou and colleagues reading lamprey sequences as one round plus smaller duplications, the dispute unresolved. It is also a different mechanism: a whole-genome duplication copies the library at once; the ligand fork copies one gene beside itself. The peptides forked locally. The receptors that read them forked globally.

The surviving receptors keep fingerprints of that history. Paré, Bortolini and colleagues found OXTR, AVPR1A and AVPR1B all keeping an ancestral two-exon structure while AVPR2 alone gained a third; and in placental mammals OXTR sits under comparatively strong purifying selection while the three vasopressin-family receptors show higher substitution rates. What they read is not really a nine-residue string, either. Six of the nine close into a ring fastened by a disulfide bond, with a three-residue tail off it, and Waltenspühl, Schöppe, Ehrenmann, Kummer and Plückthun's 2020 crystal structure of the human oxytocin receptor confirmed that the ring conformation is what recognition reads. One copy was held still; three were left to drift.

Vasopressin's own day job is unglamorous and probably older than anything social. It binds AVPR2 on the kidney's collecting-duct cells, inserting aquaporin-2 water channels into the membrane so water is pulled back out of the urine; break either end and the result is diabetes insipidus — liters of dilute urine, unrelenting thirst, a night of waking to drink. The evolutionary reading agrees, since the single ancestral gene in both outgroups is the vasopressin-type one, and where that peptide has been characterized in an invertebrate deuterostome, a sea cucumber, the job is feeding and osmoregulation. That is a working inference, not a measured date: nothing dates when social split from metabolic, only when the genes did.

Vol. I walked the rest of that menagerie — isotocin, mesotocin, vasotocin, nematocin, annetocin, conopressin — from a Panamanian rain pool to a worm burrow, and this chapter does not walk it again. What it left standing is the mammalian half: the pair bond, and the popular molecule built on it.

The fork and the myth

The prairie vole made oxytocin famous. In 1993 Winslow, Hastings, Carter, Harbaugh and Insel reported in *Nature* that central vasopressin was required for pair bonding in these monogamous rodents, and over thirty years the literature became a textbook case: blockers abolished bonds, agonists induced them, receptor maps separated monogamous voles from promiscuous relatives. Necessity and sufficiency, from converging pharmacology. Then the tools changed. Devanand Manoli's and Nirao Shah's laboratories deleted the receptor gene with CRISPR and published in *Neuron* in 2023 under a title that concedes nothing – the oxytocin receptor is not required for social attachment in prairie voles. The animals bonded anyway, tested every way the team could devise, and receptor-null mothers delivered and raised pups through weaning.

Two readings stay open. Vasopressin signaling through V1aR may compensate when the receptor is absent from conception, which a blocker dripped onto an adult brain can never test; or the necessity claim was wrong from the start. A 2024 follow-up from the same group, reporting that the receptor also shapes promiscuity and developmental timing, is a preprint awaiting peer review. Both bodies of work were done carefully, and they disagree. This is what an overturned commit looks like while it is still being overturned.

The human literature is weaker, and it is the source of the label this book will not use. Kosfeld, Heinrichs, Zak, Fischbacher and Fehr reported in *Nature* in 2005 that a nasal spray of oxytocin raised how much money strangers transferred in an economic trust game – the single empirical root of "love hormone" and "cuddle chemical." In 2015 Nave, Camerer and McCullough reviewed six replication attempts running from an increase to no effect to a decrease in trust, and flagged serious variability in the assays themselves; a registered replication in *Nature Human Behaviour* in 2020 again found no reliable effect. In the best-powered clinical test yet run – Sikich and colleagues in the *New England Journal of Medicine* in 2021, 277 children and adolescents with autism, twenty-four weeks – intranasal oxytocin was null on its primary endpoint. Oxytocin also raises

in-group favoritism, envy and out-group bias in some paradigms, which is not the behavior of a love molecule. The nasal route is no cleaner: roughly 0.001 to 0.005 percent of a dose reaches cerebrospinal fluid.

The ledger below is lopsided, and the shape is the finding. The gene history is sturdiest — two outgroup genomes, a synteny argument, a fork bracketed between two dated splits — while the birth and milk physiology, nearly as solid, carries no deep-time date at all. The behavioral rows are where the evidence thins, and the human row is the thinnest so far: a molecule whose deep past is better established than its present reputation. Peptides that hold one animal to another are not the only short chains a vertebrate inherited — the next family lets an animal ignore its own damage.

Commit Ledger

| Function | First commit | Grade | What would move the date |

|---|---|---|---|

| Ancestral nonapeptide — one vasotocin-type gene | 600–450 Mya; bracketed between two independently dated splits, not clock-dated [Vol. I anchor] | B | Resolution of the one-versus-two whole-genome-duplication dispute |

| OXT/AVP ligand fork — local tandem duplication | ~560–450 Mya; phylogenetic bracketing (jawless/jawed split as floor, elephant shark as ceiling); no clock estimate published | B | A nonapeptide locus from a jawed lineage more basal than elephant shark |

| Receptor family — OXTR plus three AVPRs | 600–450 Mya; two whole-genome duplications inferred from fourfold synteny [Vol. I anchor] | B, mechanism contested | Cyclostome resequencing settling the duplication count |

| Water balance / osmoregulation | No independent date; inferred from ancestral gene content in lamprey and amphioxus | D | A functional assay of the lamprey's own vasotocin gene, never performed |

| Birth contraction (Ferguson reflex) | Mechanism established from 1906; no deep-time date | B for mechanism | Whether the same feedback loop runs in marsupials and monotremes |

| Milk ejection (let-down) | Mechanism from twentieth-century physiology; no deep-time date | B for mechanism | Comparative lactation-hormone data outside placental mammals |

| Pair bonding (voles) | Pharmacology 1993–2005 established necessity and sufficiency; a 2023 knockout contradicts it in the same species | C — two rigorous methods disagree | A V1aR-pathway knockout crossed onto the receptor-null background |

| Human trust / social cognition | Founding result 2005; critical review 2015; registered replication 2020; no robust effect | D | A large pre-registered trial with confirmed central delivery |

Overtuned if: a genuine oxytocin-family nonapeptide gene turns up in a lamprey or hagfish genome, pushing the ligand fork below the jawless/jawed split and dissolving the argument that the ancestral molecule was vasopressin-like.

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09. The Opioid Peptides: The Pain Ledger

In Hans Kosterlitz's laboratory at the University of Aberdeen, John Hughes is working through pig brain. Speed is the whole problem. What he is hunting are peptides — short chains of amino acids — and a brain left standing digests its own peptides with its own enzymes faster than a chemist can pull them out, so the tissue has to arrive fresh and go straight into the grind. Brain in, homogenise, extract, fractionate, test, repeat, batch after batch, chasing an activity that behaves like morphine and that naloxone, the drug that does nothing but sit in opiate receptors, switches off. In 1975 Hughes and Kosterlitz, with Smith, Fothergill, Morgan and Morris, published the answer in *Nature*. Two peptides, five amino acids each, read out by dansyl-Edman sequencing and confirmed by mass spectrometry: tyrosine-glycine-glycine-phenylalanine-methionine, and the same chain ending in leucine instead. They called them the enkephalins. The brain had been making its own morphine all along.

That sentence reads backwards from how the science actually ran. Two years earlier, Pert and Snyder had found the lock before anyone had the key — stereospecific, saturable opiate binding in rat brain tissue, tagged with radioactive naloxone, with a dissociation constant of 20 nanomolar and a receptor density around 30 picomoles per gram of tissue. Their 1973 *Science* paper proved that a vertebrate nervous system contains a site built to hold an opiate. It could not say why. A receptor that binds a poppy alkaloid is either an extraordinary coincidence or evidence that the animal already makes something the alkaloid resembles. Hughes and Kosterlitz settled it: the receptor came first, and the poppy is an imitator.

The family kept growing. In 1979 Avram Goldstein's group, working from pig pituitary, isolated a third endogenous opioid — dynorphin-(1-13), roughly seven hundred times more potent than leucine-enkephalin in the guinea pig ileum assay, and fully reversed by naloxone. Sixteen years later a fourth arrived, discovered twice in the same year by two teams working independently: Meunier's group in *Nature* and Reinscheid's in *Science*,

both reporting the natural ligand of a receptor that resembled the opioid receptors but refused to bind any known opioid. It is called nociceptin. Three peptides in six years, a fourth two decades later, and — as it turned out — four separate genes behind them, not one gene read four ways.

The sequences give away the relationship immediately. Met-enkephalin and Leu-enkephalin come from the gene *PENK*; the dynorphins from *PDYN*; β -endorphin from *POMC*, a gene already famous as a pituitary stress hormone before anyone knew it was also an opioid. All three products open with the identical four residues, Tyr-Gly-Gly-Phe, before diverging. Dynorphin A does not merely resemble Leu-enkephalin — its first five residues are Leu-enkephalin itself, with the chain simply allowed to run on. β -endorphin opens with Met-enkephalin in exactly the same way. This is not three designs converging on one solution. It is one ancestral sentence, copied, and then finished differently three times.

It also explains the poppy. The receptor's binding pocket recognises a specific physical arrangement — a positively charged nitrogen held at a fixed distance and angle from an aromatic ring — and every one of these peptides presents exactly that arrangement in its first residue, tyrosine. Morphine's fused ring system happens to reproduce the same geometry closely enough to be read as the same key. The plant did not evolve to speak to vertebrates; the vertebrate receptor evolved for a message the plant's molecule imitates by accident. Nociceptin is the exception that confirms the rule. Its chain opens Phe-Gly-Gly-Phe — phenylalanine where the others carry tyrosine, a single missing hydroxyl group at position one. That one substitution is very likely why nociceptin does not activate the classical receptors, and why naloxone, a molecule built to compete with a tyrosine-anchored ligand, cannot touch the fourth receptor at all. One family, one shared opening line, and one member that lost the exact letter the others kept.

Underneath the chemistry sits an unusually clean piece of genome history. There are four peptide precursor genes — *POMC*, *PENK*, *PDYN*, *PNOC* — and four receptors: mu, delta, kappa and the nociceptin receptor, MOR, DOR, KOR and NOP. Four and four is not a coincidence.

Dan Larhammar's group at Uppsala showed in 2008 that the four receptor genes sit inside a chromosomal region carrying at least fourteen other gene families, every one of them showing the same fourfold duplication pattern — synteny, the tendency of genes to keep their neighbours across hundreds of millions of years, doing the work that sequence similarity alone cannot. That signature is the fingerprint of two rounds of whole-genome duplication at the base of the vertebrates, the same 2R event Vol. I's chapter on the bonding peptides used to date the oxytocin family. The peptide side is messier and the same group said so: their 2010 analysis puts three of the four precursor lineages on the whole-genome duplications and the POMC/PNOC separation on a smaller local duplication, on roughly the same timescale but by a different mechanism.

POMC is the strangest gene in the set. It is transcribed once and then cut apart differently in different tissues, so that a single messenger yields adrenocorticotrophic hormone, which drives the stress axis; the melanocyte-stimulating hormones, which set skin and coat colour; and β -endorphin, which relieves pain. Does and Baron trace this architecture to an ancestral opioid-coding gene that acquired its melanocortin cassette around the earlier single chordate duplication, with the second round adding a further segment on top. Stress, pigmentation and analgesia are not three genes that happen to sit near each other. They are three phrases in one continuously read sentence, separated only by which enzyme does the cutting. That is very likely why POMC survives essentially unchanged in every vertebrate lineage examined: losing it does not cost one function, it costs three at once.

The System That Arrived Whole

How far back does any of it go? The cleanest answer arrived only recently. In work published online in 2020 and carried in the journal's 2022 volume, Huang and colleagues sequenced a functional mu-opioid receptor and two enkephalin-like precursor genes directly out of the Pacific hagfish, *Eptatretus stoutii* — a jawless fish, one of the two

surviving cyclostome lineages — and found the lamprey, the other, carrying a clearly related receptor sequence. That places a working opioid system at the root of Vertebrata itself, in the neighbourhood of 550 million years ago: not a molecular-clock estimate but a bounded one, sitting below the split between jawless and jawed vertebrates that Vol. I placed at roughly 560 million years, and cross-braced by synteny and phylogeny from the other direction. The authors describe it as earlier than any previously authenticated account. What is remarkable is not the date but the shape. The system does not appear as one gene that later diversified. It appears already quadruple, already differentiated, already the four-and-four architecture a human carries — pain relief shipped as a complete package, in a single commit.

Whether anything opioid predates that root is the chapter's genuinely open question, and it is where the evidence grades earn their keep. From 1989 onward, George Stefano, with Cadet and Scharrer and a run of later collaborators, pursued a long, peer-reviewed program reporting opioid peptides and morphine-like activity in invertebrate tissue — mussel, leech, and immune cells — using immunoreactivity, chromatography and pharmacology; Laurent and colleagues reported related findings from leech ganglia in 2000. Against that stands one much newer result: the same 2020 hagfish paper ran a genome-wide search across invertebrate genomes and found no opioid receptor sequences and no opioid precursor analogues anywhere, despite deliberately looking. This does not prove the earlier data were artefacts on their own terms — an antibody that binds and a tissue that responds are real observations. It means that whatever those assays detected was very likely not a vertebrate-homologous opioid gene product. And the alternative readings are not hypothetical: morphine itself has been detected in hay, lettuce, human and cow milk, and commercial rodent feed, which is why the modern standard for claiming an animal makes its own opiate is isotope-labelled precursor tracing — watching the cell build the molecule — rather than finding the molecule present. Grade C, not D. This is a named dispute with data on both sides, not a bare hypothesis, and it is decided by a genome or not at all.

The human end of the system is less tidy than its genetics. Henning Boecker's team PET-scanned ten athletes before and after a two-hour run using a tracer that competes with the body's own opioids for receptor space; binding fell across prefrontal and orbitofrontal cortex, anterior cingulate, insula and temporoparietal regions, and the size of the fall tracked how euphoric each runner said he felt. That was the first direct image of runner's high in a living brain, and it mattered because the field's previous proxy — β -endorphin measured in blood — cannot demonstrate anything central, since peptides do not cross the blood-brain barrier efficiently. Then, in 2015, Fuss and colleagues found that in mice, blocking opioid receptors left running's anxiety- and pain-reducing effects essentially intact, while blocking cannabinoid receptors abolished them. The two results are not a direct contradiction — different species, and different halves of the phenomenon — but the popular single-molecule story does not survive meeting both. Runner's high is partly opioid at most.

The one place the receptor's role in a human is unambiguous is stranger still. In 1978, Jon Levine, Newton Gordon and Howard Fields gave patients recovering from third-molar extraction an injection of saline described as possible pain relief. Those who reported it working were then given naloxone without being told, and their pain came back. Reported in *The Lancet*, it remains the cleanest demonstration that a person can manufacture analgesia from expectation alone, running it through the same receptors morphine occupies. That fits what the endogenous opioid system appears to be for. As Richard Bodnar's long-running review series frames it, analgesia is not a standing discount on pain — pain is useful, and an animal that could not feel it would not last — but a conditional override, triggered by stress, that lets an animal finish a fight or an escape and settle the bill afterwards.

The same pocket has a darker salience. Fentanyl is a synthetic molecule that clinical pharmacology places at roughly fifty to a hundred times morphine's potency at that receptor, precisely because of how efficiently it fits. The most recent finalised United States figures record 79,384 overdose deaths in 2024 against 105,007 the year before — an age-

adjusted rate down 26.2 percent, the steepest single-year fall of the decade — with opioid-involved deaths dropping from 79,358 to 54,045 and the synthetic-opioid rate, fentanyl's category, down 35.6 percent: a real reversal in a crisis that is not over, and a figure worth re-checking against newer data before it is read as a trend. Naloxone is the thread running through the whole century: the radioactive probe that first tagged the receptor in 1973, the drug that took back a placebo's borrowed relief in 1978, and — under other names, at higher doses, carried now by paramedics and bystanders — the thing that can still displace a fentanyl molecule from a 550-million-year-old binding pocket fast enough to restart breathing.

The honest standing is lopsided in an unusual way. The genetics are as solid as anything in this book — two independent kinds of evidence, chromosomal neighbourhoods and gene trees, agreeing with a functional receptor pulled from a living jawless fish, all pointing at one moment near the vertebrate root when four peptides and four receptors came into existence together. The behaviour is softer: analgesia is confirmed in mammals and inferred in everything older, reward has a floor but not a bottom, and the one claim that would break the whole frame — a real opioid gene in a snail or a worm — has a research program behind it and no genome to show for it. That is the shape of the ledger below, and it is the eighth and last of them. Every family in this book now carries its own dates, its own grades, and its own particular way of being wrong; what is left is to lay all eight side by side and read what the log itself says.

Commit Ledger

Function	First commit	Grade	What would move the date
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Ancestral single-copy opioid precursor and receptor genes, before any duplication Imprecise. Logically required before 2R, so older than ~550 Mya, possibly tied to the single chordate duplication at 600+ Mya; inferred from the shape of the paralog tree, not observed D A			

confirmed opioid precursor or receptor ortholog in an amphioxus, other cephalochordate, or tunicate genome |

| Peptide-precursor quadruplication (POMC, PENK, PDYN, PNOC from one ancestor) | ~550 Mya, before the jawless-jawed vertebrate split; phylogeny plus synteny, cross-checked by gene presence in living cyclostomes. Three lineages from 2R, the fourth from a local duplication on the same timescale | A | A pre-vertebrate genome carrying more than one differentiated opioid precursor lineage |

| Receptor quadruplication (MOR, DOR, KOR, NOP from one ancestor) | ~550 Mya, same window; synteny across 14+ co-duplicated gene families, plus phylogeny, plus a functional receptor sequenced from a living jawless fish | A | A cephalochordate or tunicate genome carrying two or more differentiated opioid-receptor paralogs |

| Pain modulation (analgesia) as a behavioural role | Demonstrated directly in mammals from 1975; confirmed molecularly in hagfish MOR in vitro in 2020. No in vivo behavioural test spans the 550-Myr gap | B | A behavioural analgesia assay, not a binding assay, in a cyclostome or basal fish |

| Reward and place-preference role | Lower bound ≥ 450 Mya from teleost conditioned place preference, the zebrafish result Vol. I's chapter on the vertebrate bargain already reported; direct human confirmation 2008 | B | An opioid reward-circuit assay in a cyclostome or invertebrate, cleanly separated from the dopamine system |

| Nociceptin/NOP as a fourth, pharmacologically inverted paralog | Placed at the same ~550 Mya event by synteny; its function discovered twice independently in 1995 | A | Nociceptin shown to activate MOR, DOR or KOR, or to be naloxone-sensitive, under some physiological condition |

| POMC's single-transcript, three-hormone architecture (ACTH, MSH, β -endorphin) | Opioid core tied to the single duplication at the chordate base, imprecise; melanocortin cassette elaborated further at 2R | B | A cephalochordate or tunicate POMC-like gene resolving the pre-duplication state directly |

| **Contested: a protostome opioid precursor or receptor gene** | Claimed pre-2R, possibly >600 Mya, in molluscs and annelids, on immunoreactivity, chromatography and pharmacology. Zero genomic confirmation in the most recent targeted search, 2020 | **C** | A confirmed protostome opioid precursor gene, verified by synteny or robust phylogeny rather than antibody staining |

Overtured if: a confirmed opioid precursor or receptor gene turns up in any protostome genome – mollusc, annelid or arthropod – verified by chromosomal synteny or robust phylogeny rather than by immunoreactivity, which would move this family's origin off the vertebrate root and rewrite every date above it.

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10. Reading the Whole Log

Eight families, eight ledgers, sixty-odd rows of dated functions. Any engineer handed a repository like this one knows what to do next, and it is not to read another file. It is to squash the log – collapse every branch into one timeline and read the shape of the whole. Do that with this book's ledgers and something appears that no single chapter could show: the commits are not scattered. They cluster, in layers, and the layers tell a story none of the molecules tells alone.

The bottom layer is metabolism, and it is almost as old as life. Before anything said glutamate, cells were glutamate: the glutamate/ α -ketoglutarate pair was moving nitrogen through metabolism before the last universal common ancestor, around four billion years ago, and histidine's biosynthetic pathway – the assembly line behind histamine – was complete on a similar schedule. GABA enters the same way, as a by-product of glutamate's own chemistry. The future vocabulary of the brain existed first as groceries.

The next layer up is stranger: the sensors came before the signals. The protein architectures that would one day detect these molecules – the cys-loop fold behind the GABA and glycine receptors, the channel scaffold behind the glutamate receptors, the transporter fold that serotonin's reuptake pump still uses – all trace to bacteria, and none of them, at that depth, was doing the job it does in you. A resurrected ancestral receptor from the metabotropic family binds glutamate tightly despite predating any synapse that could have used the fact. The hardware was lying around for hundreds of millions of years before the software shipped. Chapter three met the sharpest version of this lesson: a sea anemone's thirty-seven "GABA receptors," seven of them cloned and tested, and not one answering to GABA. Homology names the scaffold. It does not name the word spoken through it.

Then comes the great consolidation. In the window where animals arise and radiate – crown Metazoa somewhere between 650 and 834 million

years ago, the bilaterian ancestor inside 600 to 760 — the scaffolds acquire their modern meanings, and they do it nearly all at once. The glutamate-receptor family radiates into its four subfamilies. The vesicular packaging that makes glutamate a sent signal rather than an ambient one dates to the same floor. The amine dictionary — the receptor families for dopamine, noradrenaline and its twin octopamine, the serotonin radiation — assembles by the bilaterian ancestor on the 2023 comparative genomics, though a 2026 deorphanization result would tug the monoamines a floor lower, and that dispute is live. Nearly every family chapter in this book leans on the same dating anchor there: the protostome-deuterostome split, which molecular clocks put at 642–761 million years and fossil calibration at about 558–575. Half the ledgers in this book wobble by a hundred million years on that one disagreement, and the honest reading is not to average it away but to say it plainly: the whole middle of the log is dated in rocks-versus-clocks stereo.

The fourth layer is the loudest event in the file. Around 550 to 450 million years ago, at the root of the vertebrates, two rounds of whole-genome duplication — 2R, the largest single commit in the log — copied the entire codebase twice, and the signalling systems kept the copies. The opioid system arrives wholesale here: four precursor genes and four receptors from one ancestral pair each, graded A on synteny and phylogeny together. The dopamine receptor inventory quadruples. The vasopressin-like ancestral peptide forks into vasopressin and oxytocin. The trace-amine receptors appear, the histamine H3/H4 pair splits, a sympathetic nervous system is wired down the trunk of something lamprey-shaped. Vertebrates did not invent new words. They duplicated the dictionary and let the copies drift into new meanings — and then, a hundred million years after noradrenaline, came the log's most instructive latecomer: adrenaline, whose synthesizing enzyme the jawless fishes never received. The scream travels down wiring far older than itself.

The shape of certainty

Squash the log a different way — by grade instead of by date — and a second pattern appears, and it is the one this book most wants you to take away. The A's and B's live at the bottom of the stack. The C's and D's live at the top. The pre-LUCA metabolic commits are the best-evidenced rows in the book; the receptor-family consolidations run solid B; and then, as the functions climb toward the ones that made these molecules famous — wanting, mood, trust, wakefulness — the grades thin out to D or to the ledgers' most honest entry of all, undatable as stated. Dopamine's reward-prediction computation has no confirmed date below the teleosts. Serotonin's mood-relevant role is a mechanism question wearing a timing question's clothes. Oxytocin's trust effect may not replicate at all. The pattern is not an accident of laziness; it is structural. Gene trees date parts. Behaviour is a performance of many parts, and performances leave no synteny. The log records what was written, not what it has come to mean — and the meanings, the layer we name the molecules for, are the least datable thing about them.

One family sits outside every layer, on purpose. Histamine's receptor scaffolds are ancient, but the binding of histamine itself was acquired convergently, at least three times, and its synthesizing enzyme exists as two unrelated families still being traded between bacteria by horizontal transfer. That is the control group: what a signalling molecule looks like when its history is not frozen — patchy, re-invented, borrowed. Its existence is what makes the rest of the cast remarkable. Reinvention is clearly possible. The frozen families are frozen anyway.

So the squashed log reads, bottom to top: groceries, then sensors, then packaging, then a dictionary, then a duplication that quadrupled it, then — undated, undatable, and most recent wherever it can be dated at all — everything we actually named the molecules for. Nothing in the stack was invented for a brain. The brain is the newest consumer of an API that was already old when the first neuron fired.

The log now says, as well as evidence can, when each line of the code was written. It cannot say why the file looks like this — why these eight, and

not eight others; why nothing was ever rewritten. That question has three competing answers, and they are the last chapter's whole business.

| Layer | What landed | When | Told in |

|---|---|---|---|

| Metabolites | Glutamate as nitrogen hub; histidine pathway; GABA as by-product | ~4–3.5 Gya, pre-LUCA | 02, 03, 07 |

| Sensors | Cys-loop, channel and transporter scaffolds — in bacteria, jobs unassigned | Pre-animal, mostly undatable nodes | 02, 03, 04 |

| Meanings | Receptor families radiate; vesicular packaging; the amine dictionary | ~650–560 Mya, animal-to-bilaterian floors | 02–06 |

| The great copy | 2R: opioid system whole; OT/VP fork; receptor inventories quadruple; sympathetic chain | ~560–450 Mya | 05, 06, 07, 08, 09 |

| The latecomer | Adrenaline (PNMT, jawed vertebrates only) | ~450 Mya | 06 |

| The famous jobs | Wanting, mood, trust, wakefulness, analgesia-as-experienced | Mostly undated or undatable; graded C/D throughout | 04, 05, 08, 09 |

Overtured if: the middle of the stack slides — if the placozoan and ctenophore deorphanization programs now under way land amine-specific receptors below the bilaterian line, the dictionary layer drops a floor, and the tidy sequence of scaffold-then-meaning loses its cleanest examples.

Sources

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- **2023** · Goulty, Botton-Amiot, Rosato, Sprecher & Feuda, "The monoaminergic system is a bilaterian innovation" — [Nature Communications](#)
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- **2024** · Edens, Stundl, Urrutia & Bronner, "Neural crest origin of sympathetic neurons at the dawn of vertebrates" — [Nature](#)
- Every row of the squashed log above compresses rows from the chapter ledgers, which carry the full sourcing; no date appears here that a family chapter does not source.

11. Why These Molecules

Run the morning again. The alarm at 6:12, and the histamine that answers it is riding a receptor scaffold older than the bilaterian line, though the wake-up job itself has no date outside mammals. The want of coffee arrives on receptors quadrupled 450 million years ago, doing a computation nobody has caught below the teleost fishes. The nick on your chin closes with serotonin moved by a transporter whose protein fold worked in bacteria. The fish sauce at breakfast presses on the taste side of a molecule that was moving nitrogen through metabolism four billion years back; the truck that runs the light is answered by noradrenaline through a system wired at the base of the vertebrates, with adrenaline — a hundred million years younger — arriving second; the sneeze at the gate is the control group, working a binding site invented at least three separate times; the text from someone you love lands on the derived copy of a vasopressin-like original; and the toe that stopped hurting stopped, in whatever part, through a system that shipped whole in the great duplication. Same morning. Now every moment wears a date and a grade. One question is left standing, and it is the deepest one: why these? Why is the inventory made of these eight families — and why, across six hundred million years in which everything else about animals was rebuilt again and again, was the inventory never replaced?

Chapter one left three answers on the table. The chemical-constraint answer: there was never a choice — the cast is what amino-acid chemistry offered cheaply, and no other store was open. The frozen-accident answer, Crick's phrase borrowed from Vol. I's account of the genetic code: the assignments were arbitrary, and then a thousand dependencies made them permanent. The selection answer: the cast is still here because it kept winning. The temptation is to crown one. The evidence, laid out ledger by ledger, does something more interesting: it hands each hypothesis a different layer of the stack.

Constraint owns the bottom. It is no accident that the brain's principal words are glutamate, its own decarboxylation product GABA, and a handful of amines that are each one or two cheap enzymatic steps from an amino acid the cell already stockpiles. The "go" signal is the busiest metabolite in nitrogen chemistry; the "stop" signal is what you get when you snip one carboxyl group off "go." Serotonin is a modified tryptophan, the catecholamines are a production line run on tyrosine, histamine is histidine minus the same group GABA lost. Even the bonding peptides obey a structural budget: nine amino acids in a ring, a shape so constrained that one substitution separates the water-balance hormone from the one in the labor-ward drip. Chemistry proposed a short menu, and everything on the menu got used. Where the constraint story runs out is exactly where the ledgers' grades are strongest: it explains the cast, and explains nothing about the casting.

The casting — which molecule got which job — is where the accident froze. Nothing about an indoleamine makes it a thermostat rather than an alarm; nothing about a catecholamine makes it wanting rather than rank. The proof is the book's cleanest natural experiment, met in chapter six: noradrenaline and octopamine, chemical near-twins built by one enzyme lineage, running the same alarm brief in two halves of the animal kingdom for six hundred million years — the protostomes locked onto one, the deuterostomes onto the other, and neither side ever switched. Two frozen answers to one question, standing side by side since the bilaterian ancestor. That is not constraint, because chemistry offered both to everyone. It is not ongoing selection choosing a winner, because in six hundred million years no winner was chosen. It is what an accident looks like after it freezes: arbitrary, then load-bearing, then permanent.

What the control group proves

Selection's share is subtler, and histamine is what makes it visible. The control group proves that the freeze is not a law of nature. Binding sites for histamine were re-invented at least three times; bacterial synthesizing enzymes are still being traded laterally today; the system is

a patchwork, and it works. Reinvention is chemically possible and evolutionarily survivable – which means the seven frozen families are not frozen because change is impossible. They persist either because every mutant that tampered lost (selection standing guard over the accident), or because the dependency web priced tampering out entirely (the frozen accident's own logic). The ledgers cannot fully separate those two, and this book will not pretend they can. What the ledgers do show is selection working the edges of the frozen core: duplicated receptor inventories retained and specialised after 2R, four opioid systems kept where one might have done, an analgesia circuit whose fight-through-the-injury logic is as close to an adaptive story as anything in the book. The core froze; the copies were groomed.

So the three answers are not rivals for one throne. They answer three different questions hiding under one word:

| The question under "why" | Best answer | Carried by | Standing |

|---|---|---|---|

| Why this menu of molecules? | Chemical constraint | Every family's synthesis row: one or two cheap steps from a stockpiled amino acid | Strong – the book's best-graded rows |

| Why these assignments of job to molecule? | Frozen accident | The octopamine/noradrenaline twin experiment; receptor-scaffold reuse with meanings assigned late | Strong by elimination – neither rival explains the twins |

| Why did nothing get replaced? | Selection guarding the freeze, and the freeze pricing out change – inseparable on present evidence | Histamine's proof that reinvention is possible; 2R copies groomed into specialists | Honestly split – the book's one refusal to rule |

A book that dates its claims owes you the ways it could die, and the ledgers have been accumulating them all along. Collect the movers from every chapter and they read as one standing bet against the book's own frame. A confirmed opioid precursor gene in a snail or a worm. A

functional dopamine receptor in a ctenophore. A PNMT ortholog in a lamprey, erasing adrenaline's hundred-million-year lag. Serotonin-specific signalling — own synthesis, own selective receptor, blockable behaviour — in any non-bilaterian. A cloned basal-bilaterian channel that answers to GABA itself. A shared ancestral histamine-binding motif, promoting the control group into the frozen cast. An oxytocin-family gene in a jawless fish. Any one of these lands, and a chapter of this book gets rewritten. That is not a weakness of the method. It is the method — a ledger that cannot lose is not a ledger, and the last column exists precisely so that someone, someday, can fill it in against us.

The code is still running. It ran while you read this: glutamate carrying the sentences, GABA shaping them, dopamine deciding whether you would finish the chapter, and a histamine tide already scheduling tonight's sleep. Four billion years of commits, and not one of the authors knew what the codebase would someday compute. Nature never documents her code. She leaves that to the latest processes running on it — the first ones in the whole history of the repository able to read the log, grade it, and sign their names to the places where they might be wrong.

Overtaken if: the bets start landing together — if receptors and precursors keep turning up below every floor this book has drawn, then the cast was never frozen, only thinly sampled, and the right title for this volume would have been not *The First Commits* but *The Commits We Had Found So Far*.

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- The adjudication table and the standing bets compress the chapter ledgers, which carry the full sourcing; no claim appears here that a family chapter does not source.