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EFEITO DAS AMINAS-TRAÇO EM PEIXES-ZEBRAS

UFCSPA
Universidade Federal de Ciências da Saúde de Porto Alegre

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Orientador: Prof. Dr. Murilo Sander de Abreu

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“Ninguém, a não ser quem já experimentou, pode imaginar a sedução da ciência. Em outros estudos, você vai tanto longe quanto os outros foram antes de você, e não há nada mais para saber; mas na investigação científica há alimento contínuo para descobertas e maravilhas.”

Mary Shelley, *Frankenstein*

RESUMO

Introdução: A ansiedade constitui um importante problema de saúde, podendo estar associada a prejuízos comportamentais e sociais. Nesse contexto, as aminas-traço são compostos endógenos presentes em baixas concentrações no sistema nervoso central, capazes de modular diferentes sistemas neurotransmissores. Apesar de seu potencial envolvimento na modulação de comportamentos relacionados à ansiedade e à sociabilidade, bem como de marcadores neuroquímicos e neuroendócrinos, seus efeitos ainda são pouco compreendidos. **Objetivos:** Verificar se a exposição aguda às aminas-traço clássicas (β-feniletilamina, tiramina, triptamina e octopamina) é capaz de modular o comportamento de ansiedade e sociabilidade em peixes-zebras adultos, bem como sua influência sobre alterações neuroquímicas e endócrinas. **Metodologia:** Peixes-zebras adultos foram agudamente expostos a diferentes concentrações das aminas-traço (β-feniletilamina, tiramina, triptamina e octopamina) e foram avaliados o comportamento tipo-ansiedade e a sociabilidade desses animais e determinados os níveis de acetilcolina cerebral e cortisol corporal total. **Resultados:** A exposição aguda à β-feniletilamina apresentou um perfil bifásico, na concentração de 12 µg/L, promoveu efeito ansiolítico e reduziu os níveis de acetilcolina cerebral, enquanto que a concentração de 1000 µg/L induziu comportamento ansiogênico e aumentou a concentração do cortisol corporal total. A triptamina, na concentração de 7 mg/L, induziu comportamento ansiogênico, reduziu a interação social e o e aumentou a coesão do cardume, os níveis de acetilcolina cerebral e cortisol corporal total. A tiramina, na concentração de 15 µg/L, e a octopamina, nas concentrações de 125, 500 e 1500 µg/L, também produziram efeitos ansiogênicos, além de aumentar a concentração do cortisol corporal total, sem alterações nos níveis de acetilcolina cerebral. **Conclusão:** Em conjunto, esses achados evidenciam a importância das aminas-traço na modulação de processos comportamentais, neuroquímicos e endócrinos no sistema nervoso central, demonstrando que seus efeitos são composto-específicos e associados à concentração. Os resultados reforçam a participação dessas moléculas em processos relacionados à ansiedade e a sociabilidade. Nesse contexto, o peixe-zebra se destaca como um modelo relevante para a investigação dos mecanismos associados às aminas-traço e para a triagem de potenciais alvos farmacológicos relacionados aos seus receptores cerebrais.

Palavras-chave: peixe-zebra; aminas-traço; ansiedade; comportamento; cortisol; acetilcolina.

Objetivo de Desenvolvimento Sustentável (ODS): ODS 3 – Saúde e Bem-Estar.

ABSTRACT

Introduction: Anxiety is a major health concern that may be associated with behavioral and social impairments. In this context, trace amines are endogenous compounds present at low concentrations in the central nervous system that can modulate different neurotransmitter systems. Despite their potential involvement in modulating anxiety-related and social behaviors, as well as neurochemical and neuroendocrine markers, their effects remain poorly understood. **Objectives:** To investigate whether acute exposure to classical trace amines (β-phenylethylamine, tyramine, tryptamine, and octopamine) can modulate anxiety-like behavior and sociability in adult zebrafish, as well as to assess their effects on neurochemical and endocrine parameters. **Methods:** Adult zebrafish were acutely exposed to different concentrations of trace amines (β-phenylethylamine, tyramine, tryptamine, and octopamine). Anxiety-like behavior and sociability were assessed, and brain acetylcholine levels and whole-body cortisol concentrations were determined. **Results:** Acute exposure to β-phenylethylamine produced a biphasic profile. At a concentration of 12 μg/L, it exerted an anxiolytic-like effect and reduced brain acetylcholine levels, whereas a concentration of 1000 μg/L induced anxiogenic-like behavior and increased whole-body cortisol levels. Tryptamine, at a concentration of 7 mg/L, induced anxiogenic-like behavior, reduced social interaction, and increased shoal cohesion, brain acetylcholine levels and whole-body cortisol levels. Tyramine, at a concentration of 15 μg/L, and octopamine, at concentrations of 125, 500, and 1500 μg/L, also produced anxiogenic-like effects and increased whole-body cortisol levels, without altering brain acetylcholine levels. **Conclusion:** Taken together, these findings highlight the importance of trace amines in modulating behavioral, neurochemical, and endocrine processes in the central nervous system, demonstrating that their effects are compound-specific and concentration-dependent. The results reinforce the involvement of these molecules in processes related to anxiety and sociability. In this context, zebrafish represent a relevant model for investigating the mechanisms underlying trace amine activity and for screening potential pharmacological targets associated with their receptors in the brain.

Keywords: zebrafish; trace amines; anxiety; behavior; cortisol; acetylcholine.

Sustainable Development Goal (SDG): SDG 3 – Good Health and Well-Being.

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LISTA DE ABREVIATURAS E SIGLAS

5-HT1A	Receptor serotoninérgico 5-HT1A
5-HT2A	Receptor serotoninérgico 5-HT2A
ACh	Acetilcolina
AADC	Descarboxilase de L-aminoácidos aromáticos
Acetil-CoA	Acetil-coenzima A
ACTH	Hormônio adrenocorticotrófico
AMPA	Ácido α-amino-3-hidroxi-5-metil-4-isoxazolpropiônico
CEUA	Comissão de Ética no Uso de Animais
ChAT	Colina acetiltransferase
CID-11	Classificação Internacional de Doenças, 11ª revisão
CRF	Fator liberador de corticotropina
D2	Receptor dopaminérgico D2
DBH	Dopamina β-hidroxilase
DMT	N,N-dimetiltriptamina
DSM-5	Manual Diagnóstico e Estatístico de Transtornos Mentais, 5ª edição
GPCRs	Receptores acoplados à proteína G
HHA	Eixo hipotálamo-hipófise-adrenal
HPI	Eixo hipotálamo-hipófise-interrenal
INMT	Indoletilamina N-metiltransferase
ISSN	*International Standard Serial Number*
MAO	Monoamina oxidase
MAO-A	Monoamina oxidase A
MAO-B	Monoamina oxidase B
PNMT	Feniletanolamina N-metiltransferase
SNC	Sistema Nervoso Central
TAAR1	Receptor associado a aminas-traço 1
TAAR2	Receptor associado a aminas-traço 2
TAAR5	Receptor associado a aminas-traço 5
TAAR6	Receptor associado a aminas-traço 6
TAAR8	Receptor associado a aminas-traço 8

TAAR9	Receptor associado a aminas-traço 9
TAARs	Receptores associados a aminas-traço
TH	Tirosina hidroxilase
UFCSPA	Universidade Federal de Ciências da Saúde de Porto Alegre

LISTA DE SÍMBOLOS

β	Beta
μg/L	Micrograma por litro
mg/L	Miligrama por litro
%	Porcentagem
α	Alfa
<	Menor que
ng/g	Nanograma por grama
mg/kg	Miligrama por quilograma
min	Minutos
s	Segundos

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1 INTRODUÇÃO

1.1 Ansiedade e sociabilidade

A ansiedade constitui uma resposta adaptativa que envolve alterações emocionais, cognitivas, fisiológicas e comportamentais diante de situações percebidas como ameaçadoras, acompanhadas de sensações como medo e apreensão. Entretanto, quando intensificadas, essas respostas podem desencadear uma série de sintomas associados a prejuízo funcional (1, 2). A persistência desses sintomas caracteriza os transtornos de ansiedade, que estão entre as condições psiquiátricas de maior impacto global, com uma prevalência mundial de 4,4%, e aproximadamente 10% no Brasil (3, 4). O diagnóstico de transtornos de ansiedade é baseado nas diretrizes do Manual Diagnóstico e Estatístico de Transtornos Mentais - 5 (DSM-5) e pela Classificação Internacional de Doenças - 11 (CID-11) (1, 2). Embora cada transtorno apresente características clínicas e bases neurobiológicas distintas, evidências indicam que múltiplos fatores contribuem para sua etiologia, incluindo predisposição genética, diferenças relacionadas ao sexo e fatores ambientais (1, 5). Em geral, as manifestações dos sintomas incluem dores no peito, fadiga, palpitações e distúrbios do sono, os quais comprometem atividades cotidianas, causando prejuízos no desempenho acadêmico, profissional, familiar e social (3). Apesar desse impacto e da existência de tratamentos eficazes, apenas cerca de 27,6% das pessoas com transtornos de ansiedade recebem tratamento, evidenciando também uma importante lacuna assistencial (6).

Entre os prejuízos associados à ansiedade, as alterações na sociabilidade são particularmente relevantes. O medo, a apreensão e a evitação de situações sociais podem limitar as interações interpessoais e comprometer a formação e manutenção de vínculos, especialmente em quadros como o transtorno de ansiedade social (7). Assim, a sociabilidade constitui um componente fundamental do comportamento em diversas espécies, participando da organização social, da comunicação e da adaptação ao ambiente, desempenhando um importante papel para a saúde física e mental dos indivíduos (8, 9). Assim, a redução ou perda dessas relações sociais, observadas em diferentes situações de estresse e comprometimento emocional, pode representar um prejuízo para a saúde e o bem-estar dos indivíduos (9, 10). Uma meta-análise de estudos longitudinais, envolvendo 115.133 participantes, demonstrou que maiores níveis de ansiedade social predizem posteriormente pior qualidade das relações sociais em geral (7). Por outro lado, interações e recompensas sociais positivas já demonstraram exercer efeito protetor sobre a resposta ao estresse em humanos (11-13) e em animais (14, 15). Por exemplo, em humanos, o

suporte social foi associado à redução da resposta de cortisol e da resposta subjetiva ao estresse psicossocial (13). Em chimpanzés selvagens, a presença ou interação com parceiros sociais próximos foi associada a menores níveis de glicocorticoides urinários durante situações estressoras (14).

Dessa forma, a investigação dos mecanismos neurobiológicos que regulam comportamentos relacionados à ansiedade e à interação social é de suma importância (16). Diversos sistemas neurotransmissores participam da regulação desses comportamentos, incluindo sistemas monoaminérgicos e colinérgicos (17, 18). Entre eles, a acetilcolina desempenha papel importante na modulação de processos relacionados à ansiedade, atuando em diferentes regiões cerebrais envolvidas na regulação da resposta ao estresse e do comportamento emocional (19, 20). Evidências clínicas recentes sugerem que a modulação do sistema colinérgico, particularmente por meio de receptores nicotínicos α7, pode influenciar respostas relacionadas à ansiedade em situações de estresse social (21). Em conjunto, essas evidências demonstram que a investigação dos mecanismos neurobiológicos subjacentes à regulação da ansiedade e da sociabilidade constitui uma abordagem relevante para a compreensão da fisiopatologia dessas desordens.

1.2 Aminas-traço

As aminas-traço são compostos biologicamente ativos sintetizados endogenamente em pequenas quantidades (< 10 ng/g) no encéfalo e estruturalmente se assemelham a aminas biogênicas, como serotonina e dopamina (22). Entre as principais aminas-traço destacam-se β-feniletilamina, tiramina, triptamina e octopamina (23). As aminas-traço são derivadas principalmente do metabolismo de aminoácidos aromáticos, essa descarboxilação ocorre pela ação da enzima descarboxilase de L-aminoácidos aromáticos (AADC) (24, 25). Caracterizam-se por uma rápida etapa de síntese e degradação, o que resulta em meia-vida curta e ação transitória, sendo metabolizadas pela monoamina oxidase A (MAO-A) e monoamina oxidase B (MAO-B) (26, 27). No sistema nervoso central (SNC), as aminas-traço podem atuar como neurotransmissores e, por meio da ativação de seus receptores, modular a neurotransmissão e a excitabilidade neuronal, incluindo alterações na atividade de sistemas monoaminérgicos (24). Além disso, alterações nos níveis endógenos de aminas-traço têm sido associadas a condições neuropsiquiátricas, incluindo transtornos de humor, ansiedade e disfunções cognitivas, reforçando seu potencial papel na fisiopatologia de desordens relacionadas ao estresse (24).

As aminas-traço agem por meio de seus receptores associados a aminas-traço (TAARs) específicos (22, 28, 29), que variam amplamente, de 6 em humanos (TAAR1, TAAR2, TAAR5,

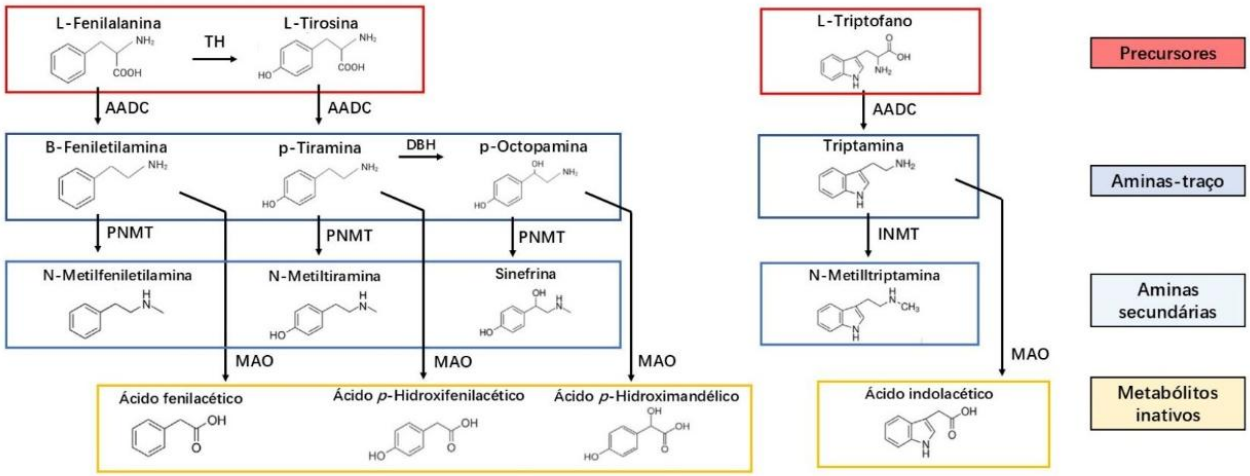
TAAR6, TAAR8 e TAAR9) a 15 em camundongos e 112 em peixes-zebras (*Danio rerio*), evidenciando a expansão desses receptores em organismos não mamíferos (24, 30). Os TAARs são uma família de receptores acoplados à proteína G (GPCRs), caracterizados pela capacidade de ativar vias de sinalização intracelular após sua estimulação (22, 27, 31). A ativação dos TAARs tem demonstrado efeitos semelhantes aos antidepressivos em modelos animais (32, 33). Os agonistas de TAAR1 (por exemplo, RO5256390) e agonistas parciais (por exemplo, RO5263397) mostraram efeitos comportamentais semelhantes a antidepressivos e antipsicóticos em roedores e primatas (34). Em camundongos submetidos à administração intracerebral do peptídeo beta-amiloide (modelo que causa déficits de aprendizagem e memória, bem como alterações comportamentais semelhantes à doença de Alzheimer), o tratamento com agonista TAAR1 (RO5256390) causou um efeito pró-cognitivo, como demonstrado no teste do labirinto em Y (35). Ainda, em camundongos, foram evidenciados efeitos terapêuticos do agonista parcial de TAAR1 RO5263397 no comprometimento cognitivo induzido por um modelo de estresse de derrota social crônico, em que animais tratados com RO5263397 tiveram a memória de reconhecimento restaurada no teste de reconhecimento de novos objetos (36).

A beta-feniletilamina é produto da descarboxilação da L-fenilalanina (Figura 1), atua via TAAR1 (24, 28) e encontra-se distribuída por todo o SNC em humanos e mamíferos (37). A capacidade da β-feniletilamina de modular rapidamente a neurotransmissão monoaminérgica tem sido evidenciada por sua interação com o receptor TAAR1, alterando a função de transportadores de dopamina, noradrenalina e serotonina e promovendo liberação destes neurotransmissores (38). Essa modulação nos circuitos dopaminérgicos e serotoninérgicos por meio de TAAR1 tem implicações em estados funcionais alterados, como os induzidos por estresse agudo ou crônico, nos quais flutuações rápidas nos níveis de monoaminas afetam sociabilidade, resposta emocional e desempenho cognitivo (28). Em roedores, é sintetizada dentro das regiões de dopamina nigroestriatal e mesolímbica (39) e evoca múltiplos efeitos comportamentais, incluindo comportamento semelhante à ansiedade, diminuição da interação social (40) e comportamento estereotipados (por exemplo, movimentos repetitivos e comportamento rotacional) (41, 42).

A triptamina é sintetizada no cérebro pela descarboxilação do L-triptofano (Figura 1) e atua via TAAR2 (22, 28, 29) e receptores serotoninérgicos (por exemplo, 5-HT1A e 5-HT2A) (43, 44), como um modulador híbrido evocando vários efeitos no SNC. Por exemplo, aumenta a atividade locomotora e vertical de ratos após injeções intra-accumbens bilaterais (45) e reverte estados semelhantes à depressão induzidos por reserpina em coelhos (46). Compostos derivados da triptamina (por exemplo, psilocibina) induzem efeitos ansiolíticos e antidepressivos em

roedores (47) e vêm sendo investigados clinicamente em protocolos assistidos para o tratamento de transtornos depressivos (44, 48, 49). Além disso, estudos recentes demonstram que o composto N,N-dimetiltriptamina (DMT), derivado diretamente da triptamina, administrado em única dose promove um aumento da neurogênese e restauração da arquitetura no giro dentado do hipocampo em camundongos adultos, o que contribuiu para reverter comportamento depressivo e déficits cognitivos (50), demonstrando que a triptamina e seus derivados como importantes moduladores da neuroplasticidade e do comportamento.

Figura 1 – Diagrama geral da bioquímica das aminas-traço



Fonte: Adaptado de Galstyan et al. (24). Nota: AADC: descarboxilase de L-aminoácidos aromáticos; DBH: dopamina β-hidroxilase; INMT: indoletilamina N-metiltransferase; MAO: monoamina oxidase; PNMT: feniletanolamina N-metiltransferase; TH: tirosina hidroxilase.

Tiramina, outra amina-traço, é produzida por meio da descarboxilação do aminoácido tirosina (Figura 1) e degradada por várias enzimas, incluindo a MAO (22, 24, 51). A tiramina tem alta afinidade para se ligar a TAAR1 e TAAR2 (22) e exibe múltiplos efeitos neuromoduladores e outros efeitos fisiológicos em humanos e animais (52, 53). A ingestão de tiramina tem sido associada ao desencadeamento de enxaqueca (53), além de alterações de humor e déficits cognitivos (54). Ademais, a octopamina é sintetizada no cérebro a partir da tiramina (Figura 1) e também desempenha um papel importante no SNC (25, 55). Por exemplo, a infusão intraventricular de octopamina evoca fenótipos semelhantes à depressão (56) e hipolocomotores em ratos (57, 58). Dessa forma, se torna evidente que estudos clínicos e pré-clínicos crescentes sugerem as aminas-traço como moduladores promissores de

comportamentos relacionados ao estresse e à ansiedade (59, 60), por sua ligação aos TAARs, resultando em uma potencial nova classe de fármacos ansiolíticos (61).

1.3 Marcadores

1.3.1 Marcador neuroquímico - Acetilcolina

A acetilcolina (ACh) é um neurotransmissor evolutivamente conservado e amplamente distribuído nos sistemas nervosos de vertebrados e invertebrados (62, 63). A ACh é sintetizada nos terminais dos neurônios colinérgicos pela enzima colina acetiltransferase (ChAT) a partir de colina e acetil-coenzima A (Acetil-CoA) (62-64). O ciclo colinérgico inclui sua biossíntese, transporte e armazenamento vesicular, liberação sináptica, interação com seus receptores e degradação pela acetilcolinesterase, com posterior reaproveitamento da colina (64). A acetilcolina atua por meio de receptores nicotínicos e muscarínicos distribuídos em diferentes regiões do SNC envolvidos no processamento das emoções, incluindo hipocampo e córtex pré-frontal (65, 66). Os receptores nicotínicos de ACh são canais iônicos ativados por ligante e estão envolvidos na transmissão sináptica rápida, além de exercerem funções moduladoras no SNC (63, 67). Já os receptores muscarínicos são receptores acoplados à proteína G, cuja ativação pode produzir efeitos inibitórios e excitatórios, dependendo do subtipo de receptor e da via de sinalização envolvida (64). A neurotransmissão colinérgica participa de diferentes processos relacionados à regulação emocional e exerce importante papel sobre a cognição. Alterações na atividade desse sistema têm sido associadas a comportamentos semelhantes à ansiedade (19-21, 68). A participação do sistema colinérgico em comportamentos relacionados à ansiedade também é observada em peixes-zebras. Nesse modelo, a inibição da acetilcolinesterase pelo donepezil foi associada ao aumento de comportamentos semelhantes à ansiedade e dos níveis de cortisol, indicando uma relação entre o aumento da sinalização colinérgica, a resposta comportamental e a ativação do eixo de estresse (69).

Evidências experimentais indicam que a β-feniletilamina pode modular a neurotransmissão colinérgica de forma indireta, por meio da ativação de circuitos glutamatérgicos e da participação de receptores dopaminérgicos D2 em ratos (70, 71). A administração de β-feniletilamina promove aumento dose-dependente da liberação de acetilcolina no estriado, como por exemplo, em doses baixas (12,5 mg/kg) observa-se ativação moderada do sistema colinérgico, enquanto doses mais elevadas (25 mg/kg) promovem uma ativação desses circuitos, efeito mediado pela ativação de receptores glutamatérgicos do tipo AMPA (70). De forma complementar, a administração sistêmica promove aumento dose-dependente da liberação de acetilcolina no estriado, com efeitos mais pronunciados em doses de 25–50 mg/kg, enquanto

doses mais baixas apresentam impacto limitado (71). Contudo, a aplicação local no estriado resulta em diminuição da liberação de acetilcolina de forma dose-dependente, efeito associado à ativação de receptores dopaminérgicos D2 (71). Esses achados indicam que a ACh é um importante neurotransmissor sobre o comportamento relacionados a ansiedade, e as aminas-traço podem modular esses comportamentos por meio de vias colinérgicas.

1.3.2 Marcador neuroendócrino - Cortisol

O estresse é uma manifestação psicobiológica amplamente estudada, caracterizada pela capacidade do organismo de perceber e antecipar ameaças físicas ou psicológicas e desencadear mecanismos adaptativos (72, 73). Essa resposta constitui um mecanismo alostático desencadeado pela percepção de um estímulo estressor, real ou potencial, permitindo ao organismo mobilizar alterações químicas, fisiológicas e comportamentais, componentes essenciais para a manutenção da sobrevivência, uma vez que permitem ao organismo lidar e superar situações adversas em curto prazo (72, 74-78).

De acordo com a duração da exposição aos estímulos estressores, o estresse pode ser classificado como agudo ou crônico, ambos podem influenciar na saúde geral do organismo (73, 74, 78). O estresse agudo refere-se a respostas de curta duração e claramente delimitadas, que geralmente induzem respostas adaptativas e processos de habituação, sendo considerado positivo quando a homeostase é restabelecida. Contudo, quando o estresse sofrido ultrapassa a capacidade do organismo em restabelecer o estado de equilíbrio, o mesmo acaba por comprometer a saúde mental e física e reduzir a qualidade de vida do indivíduo (78). Em uma situação de estresse, ocorre a ativação do eixo hipotálamo-hipófise-adrenal (HHA) em humanos (72, 75). A ativação do eixo HHA inicia-se com a liberação do fator liberador de corticotropina (CRF) pelo hipotálamo, o qual se liga aos seus receptores na hipófise anterior, estimulando a secreção do hormônio adrenocorticotrófico (ACTH). O ACTH, por sua vez, atua no córtex adrenal, promovendo a liberação de cortisol pelas glândulas adrenais. Após sua liberação, o cortisol circula pela corrente sanguínea e exerce efeitos em diversos tecidos-alvo, incluindo o cérebro, modulando respostas fisiológicas e comportamentais (75). Esse mecanismo neuroendócrino é altamente conservado ao longo da evolução dos vertebrados. Em peixes teleósteos, como o peixe-zebra (*Danio rerio*), a resposta endócrina ao estresse é mediada por um sistema análogo denominado eixo hipotálamo–hipófise–interrenal (HPI). Este eixo envolve a liberação de CRF pelo hipotálamo, secreção de ACTH pela hipófise e subsequente produção de cortisol pelas células inter-renais (79, 80).

Alterações na atividade do eixo HHA e nos níveis de cortisol têm sido associadas a comportamentos relacionados à ansiedade e à interação social. Em indivíduos diagnosticados com transtornos de ansiedade, foram observados níveis mais elevados de cortisol após o despertar quando comparados a indivíduos sem esse transtorno (81). Em contraste, pacientes com transtorno de ansiedade social apresentaram menor liberação de cortisol plasmático e salivar após exposição a um estressor social, demonstrando que exposição prolongada a um agente estressor pode atenuar a resposta do eixo HHA (82).

Alterações nos níveis de cortisol também estão associadas a respostas comportamentais relacionadas à ansiedade e a sociabilidade em peixes-zebras. Por exemplo, a exposição a um estressor agudo (tal como a perseguição por rede) aumenta o comportamento tipo-ansioso e os níveis de cortisol em peixes-zebras (83). Ademais, a exposição ao isolamento social (perda da sociabilidade) cronicamente foi associada à redução dos níveis de cortisol em peixes-zebras (84), indicando que alterações na resposta neuroendócrina ao estresse podem variar de acordo com a natureza e a duração do estímulo estressor. Por fim, os níveis de cortisol demonstram ser um importante biomarcador associado às mudanças comportamentais relacionadas à ansiedade e à interação social em humanos e peixes-zebras.

1.4 Modelo animal: Peixe-zebra

O uso de modelos animais demonstra uma importante ferramenta para elucidar os efeitos e mecanismos envolvidos nos transtornos mentais, bem como o desenvolvimento de novas terapias para essas doenças. O peixe-zebra (*Danio rerio*) tornou-se o segundo animal mais utilizado em laboratórios em todo o mundo (85), pois compartilha alta ortologia genética e fisiológica com os mamíferos (86, 87), sendo amplamente empregado como organismo modelo para o estudo da neurobiologia da ansiedade e sociabilidade, bem como na triagem de terapias para essas desordens (88, 89).

O encéfalo do peixe-zebra apresenta semelhanças neuroanatômicas e funcionais com o de mamíferos (90, 91), incluindo a conservação de componentes neuroquímicos (92). Por exemplo, a espécie possui os principais sistemas neurotransmissores e seus respectivos receptores (93, 94), abrangendo monoaminas (92, 95) e aminas-traço, como β-feniletilamina, tiramina, triptamina e octopamina (24). No peixe-zebra, a família de receptores associados a aminas-traço (TAARs) é particularmente expandida, com 112 TAARs descritos. Destes, 27 apresentam características estruturais associadas à ligação de monoaminas, aspecto também observado em todos os TAARs humanos e na maioria dos TAARs murinos (29). Além disso, o peixe-zebra

possui genes ortólogos relacionados ao TAAR1 de mamíferos, e este receptor encontra-se abundantemente expresso em diferentes regiões do SNC (24). Essa conservação estrutural e neuroquímica se manifesta em padrões comportamentais, levando em consideração que, estressores de natureza social, como alteração na composição do cardume ou isolamento social, também podem afetar as respostas comportamentais, neuroquímicas e neuroendócrinas do peixe-zebra (96). Por exemplo, a exposição aguda a um agente estressor aumenta os níveis de cortisol, desencadeia um comportamento tipo-ansioso (97) e diminui a interação social (98) em peixes-zebras. Nesse contexto, entre os aspectos fisiológicos e endócrinos, o peixe-zebra demonstra ser um importante modelo para avaliar a modulação comportamental, neuroquímica e endócrina das aminas-traço.

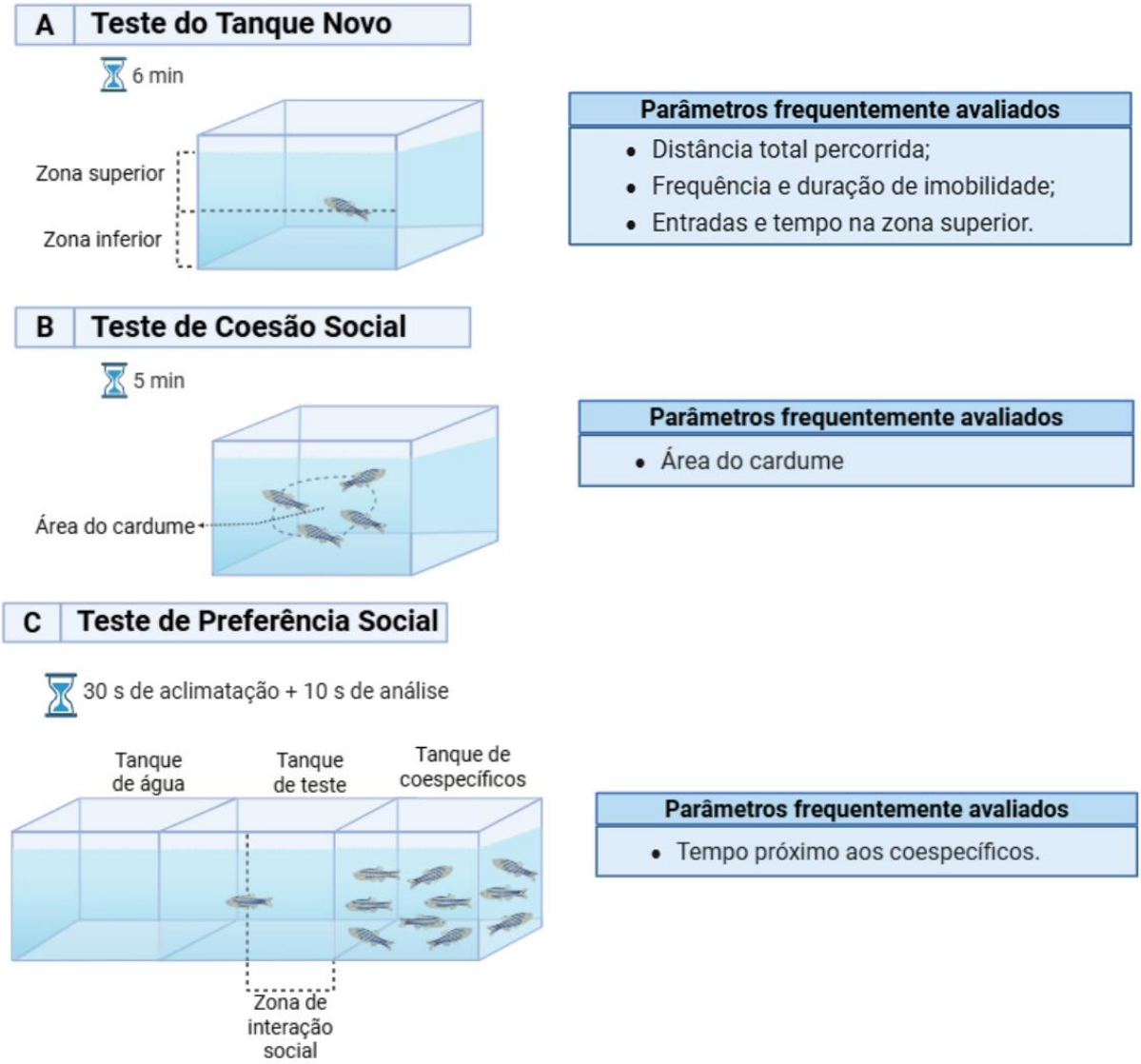
1.4.1 Testes comportamentais

Os testes comportamentais usados em peixes-zebras (*Danio rerio*) são amplamente descritos, padronizados e validados pela comunidade científica (99, 100). Esses paradigmas comportamentais apresentam boa reprodutibilidade e validade preditiva, sendo responsivos a fármacos com eficácia clínica em humanos, o que reforça sua relevância translacional (98). Os comportamentos semelhantes à ansiedade são avaliados principalmente por meio do teste do tanque novo (101). O teste do tanque novo baseia se na premissa de que quando os peixes-zebras são expostos a um ambiente "novo" (Figura 2), os animais tendem inicialmente a permanecer na região inferior do aquário, e conforme se a habituam a esse novo ambiente, começam a explorar o ambiente por completo, que inclui a exploração da região superior do aquário (102, 103). Nesse teste, os parâmetros comumente avaliados incluem a distância percorrida, episódios e tempo de imobilidade, e entradas e tempo na região superior do aquário (98, 103). Esse paradigma comportamental é sensível a manipulações farmacológicas, por exemplo, peixes-zebras expostos agudamente à fluoxetina aumentam o tempo de permanência na região superior do aquário, identificado como um efeito tipo-ansiolítico (104). Em contraste, a exposição aguda à cafeína, aumenta a permanência do animal na região inferior e reduz sua exploração no aquário, indicando um efeito tipo-ansiogênico (97).

Nesse contexto, também pode ser utilizado o teste de coesão social, no qual os peixes-zebra são avaliados em cardume (composto por 4 ou 6 animais) (Figura 2). Nesse teste, a coesão do grupo é geralmente quantificada por medidas como distância média interindividual, área ocupada pelo cardume e dispersão espacial, parâmetros que permitem inferir mudanças na motivação social e comportamento tipo-ansioso (69, 101, 105). Por exemplo, peixes-zebras quando expostos a um estressor agudamente, apresentam uma maior coesão do cardume,

caracterizada pela redução da distância entre os indivíduos, sendo associado como um comportamento tipo-ansioso (105). Similarmente, a exposição aguda à cafeína também aumenta a coesão do cardume e diminui a distância média entre os animais, demonstrando seu efeito ansiogênico em peixes-zebras (106).

Figura 2 – Representação esquemática dos testes comportamentais utilizados para avaliação de ansiedade e sociabilidade em peixes-zebras e dos principais parâmetros frequentemente avaliados.



Fonte: Figura elaborada pela autora com auxílio da plataforma BioRender.com (2026). **Nota: (A)** Teste do tanque novo: após o tratamento com os compostos, os peixes são transferidos para um aquário novo, no qual são avaliados parâmetros relacionados ao comportamento tipo-ansioso. **(B)** Teste de coesão social: após o tratamento, grupos de peixes são transferidos para o aquário de teste para avaliação de parâmetros relacionados à coesão do cardume. **(C)** Teste de preferência social: após o tratamento, o peixe é avaliado em relação ao seu posicionamento

no aquário, avaliando sua aproximação ao aquário contendo coespecíficos ou ao aquário adjacente, permitindo assim a análise da interação social.

A sociabilidade pode ser avaliada pelo teste de preferência social (Figura 2), no qual os peixes são transferidos individualmente para o tanque teste posicionado entre dois tanques, um contendo somente com água e outro contendo um grupo de coespecíficos (98). O peixe-zebra é uma espécie gregária (107) que apresenta tendência natural à formação de grupos e à aproximação de coespecíficos (105, 108). Assim, a redução na interação social (tempo gasto próximo ao aquário com seus coespecíficos) pode indicar prejuízo na interação social (101, 108). Por fim, a análise comportamental em peixes-zebras vem sendo aprimorada e permitindo uma adequada reprodutibilidade de seus ensaios.

2 JUSTIFICATIVA

O desenvolvimento de novas abordagens farmacológicas para o tratamento de transtornos do SNC constitui uma demanda clínica relevante, especialmente diante das limitações terapêuticas observadas em condições neuropsiquiátricas, como a ansiedade (50). Recentes avanços nesse campo incluem o uso de aminas-traço para o tratamento de transtornos mentais (22), particularmente devido à sua atuação sobre TAARs (27). Nesse âmbito, o uso de modelos animais é uma ferramenta fundamental para compreender os comportamentos e os mecanismos neurobiológicos e neuroquímicos envolvidos nos transtornos mentais, bem como para auxiliar no desenvolvimento de novas terapias para essas doenças. O peixe-zebra consolidou-se como um dos principais organismos modelo na pesquisa biomédica e na triagem de terapias para essas desordens, reforçando seu valor translacional (85, 88, 89).

Apesar do crescente interesse nas aminas-traço e nos TAARs, ainda há lacunas a serem preenchidas e são limitados os estudos que investigam sistematicamente os efeitos comportamentais e neuroquímicos dessas moléculas. Considerando a relevância do peixe-zebra como modelo experimental, torna-se importante investigar como aminas-traço clássicas (β-feniletilamina, triptamina, tiramina e octopamina) modulam comportamentos semelhantes à ansiedade e a sociabilidade, bem como marcadores neuroquímicos e neuroendócrinos.

3 OBJETIVOS

3.1 Objetivo geral

Verificar se a exposição aguda às aminas-traço clássicas (β-feniletilamina, tiramina, triptamina e octopamina) é capaz de modular o comportamento de ansiedade e preferência social em peixes-zebras adultos, além de parâmetros neuroquímicos e endócrinos.

3.2 Objetivos específicos

- Verificar se as aminas-traço (β-feniletilamina, tiramina, triptamina e octopamina) modulam o comportamento tipo-ansiedade em peixes-zebras.
- Avaliar o impacto dessas aminas-traço sobre parâmetros comportamentais de preferência social e coesão social.
- Analisar os efeitos das aminas-traço sobre marcadores neuroquímicos (acetilcolina) e endócrinos (cortisol) em peixes-zebras.

4 ARTIGO CIENTÍFICO

O artigo "*Acute Effects of Four Major Trace Amines on Zebrafish Behavioral, Neurochemical, and Neuroendocrine Responses*" publicado no periódico *Journal of Neurochemistry* (ISSN 0022-3042, fator de impacto 4.6, 2026), é apresentado a seguir. O estudo avaliou os efeitos agudos das aminas-traço (beta-feniletilamina, triptamina, tiramina e octopamina) sobre o comportamento do tipo-ansioso e social em peixes-zebras, bem como sobre os níveis de acetilcolina cerebral e cortisol corporal total. A beta-feniletilamina, em baixa concentração provocou efeitos tipo-ansiolítico e reduziu os níveis cerebrais de acetilcolina e em alta concentração aumentou o comportamento tipo-ansioso e os níveis de cortisol. A exposição aguda à triptamina induziu um efeito ansiogênico, reduziu a interação social, e elevou os níveis cerebrais de acetilcolina e de cortisol corporal. A exposição aguda à tiramina e à octopamina causaram efeitos ansiogênicos, acompanhados pelo aumento do cortisol. Em Anexo a carta de aprovação da CEUA da UFCSPA (Anexo A) e a autorização de cópia, reprodução ou compartilhamento do artigo publicado (Anexo B).

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Acute Effects of Four Major Trace Amines on Zebrafish Behavioral, Neurochemical, and Neuroendocrine Responses

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ABSTRACT

Trace amines are biologically active compounds endogenously synthesized in the brain in small amounts and structurally resembling biogenic amines. Acting via specific trace amine-associated receptors (TAARs), they induce robust behavioral and physiological effects in humans and animals. However, although TAAR ligands have recently been suggested as novel putative anxiolytics, their central effects and evolutionary conservation of activity remain poorly understood. Here, we evaluated the acute effects of four major trace amines (beta-phenylethylamine, tryptamine, tyramine, and octopamine) on zebrafish anxiety-like and social (shoaling) behavior, as well as neurochemical and neuroendocrine (cortisol) responses. Beta-phenylethylamine, at a low concentration (12 μg/L), caused overt anxiolytic-like effects and reduced brain acetylcholine levels; at a high concentration (1000 μg/L) increased zebrafish anxiety-like behavior and whole-body cortisol levels. Acute tryptamine exposure (7 mg/L) evoked an anxiogenic-like effect, reduced shoaling and social interaction, and elevated brain acetylcholine and whole-body cortisol. Acute exposure to tyramine (15 μg/L) and octopamine (125, 500, and 1500 μg/L) induced similar anxiogenic-like effects, accompanied by increased whole-body cortisol without altering brain acetylcholine levels. Collectively, these findings not only emphasize the important role of trace amines in brain and behavior but support the growing complexity of their CNS effects in vivo across taxa and highlight the relevance of zebrafish models for drug screening based on targeting brain TAARs.

Abbreviations: AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; CNS, central nervous system; DMSO, dimethyl sulfoxide; ESI, electrospray ionization; KW, Kruskal-Wallis (test); LC–MS/MS, liquid chromatography-mass spectrometry/mass spectrometry; MAO, monoamine oxidase; MRM, multiple reaction monitoring; PBS, phosphate buffered saline; RRID, Research Resource Identifier; TAARs, trace amine-associated receptors; TrkB, tropomyosin receptor kinase B.

Thalia M. Quintanilha and Pietra M. Costa shared first authorship.

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1 | Introduction

Trace amines are biologically active compounds that are endogenously synthesized in small (< 10 ng/g) amounts in the brain and structurally resemble biogenic amines, such as serotonin and dopamine (Fehler et al. 2010; Lindemann and Hoener 2005; Gainetdinov et al. 2018). P-tyramine, β-phenylethylamine, tryptamine, and octopamine represent common trace amines (Gainetdinov et al. 2018). The trace amines act via their specific trace amine-associated receptors (TAARs) (Gainetdinov et al. 2018; Borowsky et al. 2001; Bunzow et al. 2001), which vary widely, from 6 in humans (TAAR1, TAAR2, TAAR5, TAAR6, TAAR8, and TAAR9) to 15 in mice and 112 in zebrafish (*Danio rerio*) (Li et al. 2015; Galstyan, Krotova, et al. 2025).

Beta-phenylethylamine acts via TAAR1 (Borowsky et al. 2001) and is widespread throughout the central nervous system (CNS) in humans (Philips et al. 1978) and mammals (Durden et al. 1973). In rodents, it is synthesized within nigrostriatal and mesolimbic dopamine regions (Paterson et al. 1990) and evokes multiple behavioral effects, including anxiety-like behavior, decreased social interaction (Lapin 1990) and stereotyped behavior (Borison et al. 1977; Ortmann et al. 1984; Barroso and Rodriguez 1996) (also see similar primate findings; Tinklenberg et al. 1978). Tryptamine is synthesized in the brain by decarboxylation of L-tryptophan and acts via TAAR2 (Gainetdinov et al. 2018; Borowsky et al. 2001; Bunzow et al. 2001) and serotonergic (e.g., 5HT1A and 5HT2A) receptors (Fantegrossi et al. 2008; Woolley and Shaw 1962; Kargbo 2023), evoking various CNS effects. For example, it increases rat locomotor and vertical activity following bilateral intra-accumbal injections (Marien et al. 1987) and reverses reserpine-induced depression-like states in rabbits (Tedeschi et al. 1959). Tryptamine-based drug molecules (e.g., psilocybin) induce anxiolytic- and antidepressant-like effects in rodents (Sekssaoui et al. 2024) and have recently been approved for treatment-resistant depression and major depression clinically (Kargbo 2023; Raison et al. 2023; Goodwin et al. 2022).

Tyramine, another common trace amine, is produced via the decarboxylation of tyrosine and degraded by several enzymes, including monoamine oxidase (MAO) (Gillman 2018; Andersen 1977). Tyramine has high affinity for binding TAAR1 and TAAR2 (Gainetdinov et al. 2018) and exhibits multiple neuromodulatory and other physiological effects in humans and animals (Andersen 1977; Nagaya et al. 2002). For example, its intake is associated with migraine onset (Costa and Glória 2003) and with mood and cognitive deficits and switch from hypomania to depression, suggesting that affective behaviors may be particularly susceptible to this trace amine (Pickar et al. 1979). In turn, octopamine is synthesized in the brain from tyramine and also plays an important CNS role (Berry 2004; Burchett and Hicks 2006; Axelrod and Saavedra 1977). For example, octopamine intraventricular infusion evokes rat depression-like (Chance et al. 1985) and hypolocomotor phenotypes (Delacour and Guenaire 1983; David et al. 1982). Aberrant tyramine and octopamine neurotransmission has also been associated with a wide range of clinical CNS disorders (Zucchi et al. 2006), as they are both reduced in depression (Sandler et al. 1979), whereas tyramine levels are elevated in Parkinson's patients (D'Andrea et al. 2019), and octopamine levels are lower in patients with encephalopathy (Cuilleret et al. 1980).

Mounting clinical and preclinical studies suggest trace amines as promising modulators of stress- and anxiety-related behaviors (Wolinsky et al. 2007; Rutigliano et al. 2019; Liu et al. 2024) which can be corrected by TAARs ligands, a recently proposed new putative class of anxiolytic drugs (Alnefeesi et al. 2024). In addition to monoaminergic and trace-aminergic signaling, the central cholinergic system potently modulates various brain functions, including anxiety- and depression-like behavior (Mineur et al. 2018, 2013, 2022). For example, depressed patients have higher brain levels of acetylcholine than healthy controls (Hannestad et al. 2013; Esterlis et al. 2013), whereas stressed rats display elevated hippocampal acetylcholine (Mark et al. 1996; Mineur et al. 2022). Elevating acetylcholine levels by inhibiting hippocampal acetylcholinesterase in mice induces anxiety-like and depression-like behaviors (Mineur et al. 2013), whereas oxotremorine (a muscarinic acetylcholine receptor agonist) attenuates anxiety induced by chronic stress (Di Liberto et al. 2017).

Complementing rodent models, the zebrafish (*Danio rerio*) is rapidly becoming a powerful model species in neuroscience and neurochemistry (Costa et al. 2023), including trace amine- (Galstyan, Krotova, et al. 2025) and cholinergic neurotransmission research (Mueller et al. 2004; Clemente et al. 2004). For instance, zebrafish have 20%–48% orthologous genes, similar to those of mammalian TAAR1 receptors (Grandy 2007). Recent studies have demonstrated CNS effects of novel trace amine-like molecules in zebrafish models, as acute 24H-NBOMe(F) and 34H-NBOMe(F) treatment reduce zebrafish despair-like behavior (Demin et al. 2022), while chronic exposure to 24H-NBOMe(F) and 34H-NBOMe(F) evokes anxiolytic-like effects in the novel tank test with lowered brain norepinephrine levels, further supporting the potential of zebrafish for preclinical screening of small psychoactive trace amine-related molecules (Ilyin et al. 2024). Likewise, although acute exposure to cholinergic drugs nicotine, varenicline, and arecoline produces anxiolytic-like effects in zebrafish (Serikuly et al. 2020; Levin et al. 2007), donepezil (an acetylcholinesterase inhibitor facilitating acetylcholine action) increases anxiety-like behavior and whole-body cortisol levels (Giacomini, Bueno, et al. 2020).

In addition to high genetic and physiological homology to humans in general (Howe et al. 2013; Briggs 2002), zebrafish possess evolutionarily conserved, shared neuroanatomy and neurochemistry of both the trace amine- (Galstyan, Krotova, et al. 2025) and cholinergic systems (Mueller et al. 2004), and may therefore be used to assess the role of both systems and their interplay in stress and stress-related behavioral deficits. Recognizing the growing value of zebrafish models for studying the trace-aminergic and cholinergic modulation of CNS functions and complex behaviors, here we examined the effects of four common trace amines (β-phenylethylamine, tryptamine, tyramine, and octopamine) on zebrafish anxiety-like and social behaviors, central acetylcholine levels, and whole-body cortisol.

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2 | Methods

2.1 | Animals

A total of 284 male mature adults (4–6 months old) zebrafish of the wild-type short-fin outbred strain were obtained from a commercial supplier (Delphis, Porto Alegre, Brazil). Fish were acclimated to the laboratory environment for 1 month and housed (3 fish/L) in 13-L tanks (15 width × 35 depth × 25 height, cm) equipped with external biological filters (Ocean Tech HF-100, Brazil) under constant aeration and a light/dark (14 h light:10 h dark) cycle, with lights on at 7:00 am. Water temperature was maintained at 27.2°C ± 0.5°C; pH 6.8 ± 0.15, with dissolved oxygen kept at 6 ± 0.15 mg/L, total ammonia at < 0.01 mg/L, according to established standards of zebrafish husbandry (Avdesh et al. 2012). All fish were fed twice a day with commercial flake food (Alcon Basic, MEP 200 Complex, Brazil), following accepted standards of zebrafish husbandry. The selection of outbred fish for this study was based on the relevance of translational research to better mimic the genetic heterogeneity of clinical populations (de Abreu et al. 2022). Animal experimentation reported here was approved by the Institutional Animal Care and Use Committee of the Federal University of Health Sciences of Porto Alegre (Ref. 816/2024) and fully adhered to national and international guidelines on animal experimentation and care and principles of ethical experimentation (de Abreu et al. 2019). The experimental design, data analysis, and presentation fully adhered to the ARRIVE 2.0 (Animal Research: Reporting of In Vivo Experiments) guidelines (Percie du Sert et al. 2020) for reporting animal research and the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines (Smith et al. 2018) for planning animal research and testing. The methods were presented following the Research Resource Identifiers (RRIDs).

2.2 | Experimental Procedures

2.2.1 | Acute Effects of β-Phenylethylamine and Tryptamine

Experiment 1 examined the acute effects of β-phenylethylamine (CAS: 64-04-0, Sigma-Aldrich, St. Louis, USA) on zebrafish anxiety-like behavior. Using an online randomization tool (www.randomizer.org), zebrafish ($n = 13$ per group) were randomly allocated into 5 experimental groups: control (intact water, unexposed) and β-phenylethylamine-treated (at 12, 120, 300, and 1000 μg/L) via water immersion for 1 h. The 1 h treatment time and immersion administration were selected based on our previous studies on anxiolytic drugs in adult zebrafish (Giacomini et al. 2021; Giacomini, Piassetta, et al. 2020). The concentrations of β-phenylethylamine used here were selected based on half maximal effective concentration (EC_{50} = ~0.3 μM, ~36 μg/L) for activation of the TAAR12 receptor in zebrafish (Li et al. 2015) and TAAR1 in humans (Lindemann et al. 2005), as well as on our pilot study with this drug. The sample size ($n = 13$) in this study, necessary to obtain reliable data, was estimated based on our previous experience screening drugs in adult zebrafish (Giacomini et al. 2021), as well as prior calculations using the G*Power 3.1 program (Heinrich Heine University Düsseldorf, Germany) for one-way analysis of variance (ANOVA), effect size of 0.5, alpha of 0.05, power of 0.9, 5 groups, and a total $n = 70$.

Since the novel tank test identified the 12 μg/L concentration of the drug as inducing anxiolytic-like behavior, we assessed brain levels of acetylcholine, as well as whole-body cortisol levels. In addition, as the 1000 μg/L concentration of β-phenylethylamine demonstrated an anxiogenic-like (e.g., increasing freezing duration), an additional study was performed to examine this aspect in detail, assessing zebrafish behavior in an anxiety-sensitive shoaling test and social interaction assay. For this, a separate cohort of zebrafish from the same batch was randomly allocated into control and β-phenylethylamine-treated (at 1000 μg/L) groups ($n = 4$). The fish were initially treated in the group (4 animals each) for 1 h and then tested in the shoaling test for 5 min, and subsequently individually recorded for 10 s in the social interaction assay (see further). The group size (4 fish shoal) in this study, necessary to obtain reliable data, was estimated based on our previous experience screening drugs in adult zebrafish (Giacomini, Bueno, et al. 2020). At the end of behavioral analyses, zebrafish were euthanized in ice-cold water (with decapitation after the cessation of opercular movements for > 30 s), and their brains were briefly extracted on ice to assess acetylcholine brain levels. The headless bodies were used to reconfirm sexing by gonadal dissection under a PZ0-Labimex stereomicroscope (Metrimpex, Budapest, Hungary) and later stored at −80°C in an ultra-freezer for whole-body cortisol analyses.

Experiment 2 examined the acute effects of tryptamine (CAS: 61-54-1, Sigma-Aldrich) on zebrafish anxiety-like behavior and physiology. Zebrafish ($n = 12$ per group) were randomly allocated into 4 experimental groups: control (dimethyl sulfoxide/DMSO 0.011% vol/vol) and tryptamine-treated (at 1.5, 7, and 15 mg/L) via water immersion for 1 h. The concentrations of tryptamine used here were selected based on half maximal effective concentration (EC_{50} = ~70 μM, ~11.21 mg/L) for activation of TAAR10a in zebrafish (Li et al. 2015). We also performed a pilot study to verify whether the concentration of DMSO 0.011% used in the control group would influence the zebrafish anxiety-like behavior. As no difference was found between control (DMSO 0.011%) and intact control groups, and compliant with the reduction of animal usage as per 3Rs principles of human bioexperimentation, we used the DMSO 0.011% group as control for all experiments. Similarly, to Experiment 1, zebrafish were individually exposed to 800 mL tryptamine or vehicle for 1 h in the treatment tanks, followed by assessing their behavior in the novel tank test for 6 min.

Since this assay identified the 7 mg/L concentration of the drug as behaviorally active, producing an anxiogenic-like effect, we conducted an additional study to explore this further by assessing zebrafish behavior in the shoaling and social interaction tests. For this, a separate cohort of zebrafish from the same batch was randomly allocated into control (DMSO 0.011%) and tryptamine-treated (7 mg/L) groups ($n = 6$). The fish were initially treated in the 4-fish group for 1 h, tested in the shoaling test for 5 min, and then subsequently individually recorded for 10 s in the social interaction assay (see further). The group size here was estimated based on our previous experience screening drugs in adult zebrafish (Giacomini, Bueno, et al. 2020). As in Experiment 1, at the end of behavioral analyses, zebrafish were

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euthanized in ice-cold water and their brains were briefly extracted on ice to assess brain acetylcholine levels and whole-body cortisol, as well as to reconfirm sexing by gonadal dissection.

2.2.2 | Acute Effects of Octopamine and Tyramine

Experiment 3 examined the acute effects of tyramine (CAS: 51-67-2) and octopamine hydrochloride (CAS: 770-05-8, Sigma-Aldrich) on zebrafish anxiety-like behavior and physiology. Zebrafish ($n = 13$ per group) were randomly allocated into 7 experimental groups: control (intact water, unexposed), treated with tyramine (at 5, 15, and 135 μg/L) and with octopamine (at 125, 500, and 1500 μg/L) via water immersion for 1 h. The concentrations of tyramine used here were selected based on half maximal effective concentration (EC_{50} = ~1 μM, ~137.18 μg/L) for the activation of human and mouse TAAR1 (Lindemann et al. 2005), and the concentrations of octopamine were based on EC_{50} of ~10 μM (~1.89 mg/L) for human TAAR1 (Lindemann et al. 2005). As in Experiment 1 and 2, at the end of behavioral analyses, zebrafish were euthanized in ice-cold water to assess whole-body cortisol and to reconfirm sexing by gonadal dissection.

The respective trace amines levels in exposure solution samples were reconfirmed by liquid chromatographic system coupled to a triple quadrupole mass spectrometer (LC–MS/MS), model LCMS-8045 (Shimadzu, Kyoto, Japan), showing the actual concentrations of β-phenylethylamine 12 μg/L (12.3 ± 3.22 μg/L), 120 μg/L (126 ± 11 μg/L), 300 μg/L (280 ± 44 μg/L), and 1000 μg/L (1004 ± 114 μg/L), tryptamine 1.5 mg/L (1.53 ± 0.11 μg/L), 7 mg/L (7 ± 0.22 mg/L), 15 mg/L (15.57 ± 0.43 mg/L), tyramine 5 μg/L (5.1 ± 0.6 μg/L), 15 μg/L (14.4 ± 1 μg/L), 135 μg/L (135.8 ± 0.8 μg/L) and octopamine 125 μg/L (123.1 ± 6.66 μg/L), 500 μg/L (495 ± 54.8 μg/L), 1500 μg/L (1512 ± 134.5 μg/L).

2.3 | Behavioral Testing

Behavioral testing was performed in the animal holding room (to avoid transfer and manipulation stress) between 8.00 am and 11.00 am, recording fish behaviors by C920 cameras (Logitech Inc., Romanel-sur-Morges, Switzerland). On the day of the experiment, the fish remained unfed to avoid a concomitant effect of feeding on behavioral endpoints and were randomly allocated to behavioral testing tanks to avoid experimental bias. Behavioral, neurochemical, and endocrine assays, as well as subsequent offline data analyses, were performed by investigators blinded to the experimental groups (each tank was coded by a researcher who did not participate in the experiments).

Zebrafish were individually exposed for 1 h to treatment in the glass tank (10 width × 10 depth × 15 height, cm) with 800 mL of drug or vehicle solution prior to assessing their behavioral response in the novel tank test for 6 min. Its apparatus was a glass tank (24 width × 8 depth × 20 height, cm) filled with facility water to a height of 18 cm. During the test, we recorded zebrafish individually with a C920 camera and subsequently analyzed data offline using ANY-maze software (Stoelting Co, Wood Dale, USA), assessing the total distance traveled, freezing frequency (immobility > 2 s) and duration, as well as the time spent at the top of the tank, based on the body center position, as in (Kysil et al. 2017).

Fish were also treated with drugs or vehicle in 4 fish groups for 1 h, tested in the shoaling test for 5 min, after which they were individually assessed in the social interaction test. For the latter test, fish were transferred individually to the glass testing tank (30 width × 10 depth × 15 height, cm), positioned between two other tanks, one empty and the other containing a group of 15 conspecifics (Giacomini et al. 2016). In this test, fish acclimated for 30 s to the tank, and their behavior was then recorded for 10 s, with a subsequent off-line analysis using the ANY-maze software to assess the time spent near conspecifics. Shoaling test videos were further analyzed using the Image-J-1.49 software (National Institutes of Health, NIH, Bethesda, USA), and the shoaling phenotypes were assessed using screenshots made every 20 s over the test period (with a total of 15 screens shots per group), to calculate the dispersion of the group as the shoal size (Canzian et al. 2017). At the end of behavioral analyses, zebrafish were euthanized in ice-cold water (followed by decapitation after the cessation of breathing movements for > 30 s) and their brains quickly extracted on ice for neurochemical analyses, and headless bodies—for whole-body cortisol analyses and to reconfirm sexing by gonadal dissection under the microscope. The whole-brain and whole-body samples were stored in the ultra-freezer at −80°C for further analyses.

2.4 | Brain Acetylcholine Analyses

Brain samples were thawed, weighed, placed in test tubes with 10 μL of 0.1% formic acid solution per 1 mg/brain weight, and homogenized in the microtube with a plastic pestle for 1 min. In 50 μL of homogenized sample, we added 930 μL of pure acetone and 20 μL of the *d*-dopamine standard (250 ng/mL, Sigma-Aldrich) and centrifuged for 6 min (at 9000 rpm, 5610 rcf), after which the supernatant was placed to evaporate in the laminar flow hood until full evaporation and stored at −80°C in the ultra-freezer. Neurochemical analyses were performed using the liquid chromatographic system coupled to an 8045-Shimadzu triple quadrupole mass spectrometer (LC–MS/MS, Shimadzu Inc., Kyoto, Japan). On the day of analyses, brain samples were resuspended in ultrapure H_2O (50 μL), and the mass spectrometer was operated using electrospray ionization (ESI), with the acquisition of all analytes made in the positive mode and settings used for analyses as heat block temperature 400°C, capillary voltage 4.5 kV, nebulizer gas (N_2) flow 1.7 L/min, drying gas (N_2) flow 10 L/min, desolvation line temperature 250°C, and collision-induced dissociation gas pressure 230 kPa. Multiple reaction monitoring (MRM) mode was used for data acquisition, with two MRM transitions for each analyte—one for quantification and the other for qualification. The MRM transitions (m/z) and collision energy (V) were: acetylcholine (147.1–84.0/17 and 147.1–130.1/20).

For neurochemical analyses, the chromatographic separation was performed with a Shimpack GISS C18 column (100 × 2.1 mm, 1.9-μm particle size) from Shimadzu Inc. (Japan). The mobile phases were composed of (A) ultra-pure water with formic acid (0.1%, *v/v*) and (B) acetonitrile with formic acid (0.1%, *v/v*). The column was heated at 30°C, with a mobile phase flow of 0.3 mL/

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min. The chromatographic run was performed using a gradient as follows: 0–1 min, 0% of B; 1–6 min, 0%–100% of B; 6–7 min, 100% of B; 7–7.1 min, 100%–0% of B; 7.1–15 min, 5% of B, totaling 15 min. All data were collected and analyzed using the LabSolutions software (Shimadzu Inc.).

2.5 | Whole Body-Cortisol Analysis

Fish were homogenized by a Dremel-300 tissue homogenizer (Dremel Inc., Rancine, USA) in phosphate-buffered saline (0.5 g weight in 2 mL PBS g) for 1 min for their whole-body cortisol extraction with diethyl ether, as described previously (de Abreu et al. 2014). Cortisol levels were analyzed using a commercially available cortisol enzyme-linked immunosorbent assay kit (ELISA, Monobind Inc., Lake Forest, USA) for human serum or plasma (DCO: 1353, product code: 3625-300). The detection accuracy was tested by calculating the recoveries from standard samples containing 1, 4, 10, 20, and 50 ng/mL cortisol, with a mean detection of 95%. All cortisol values were adjusted for recovery (cortisol value = measured value × 1.05) and normalized based on the weight of the respective whole-body samples, expressed as absolute cortisol concentrations (ng/g body weight).

2.6 | Data Analysis

Data normality was assessed by the Kolmogorov-Smirnov test. In all experiments, the novel tank data were analyzed by the Kruskal-Wallis (KW) test, followed by Dunn's post hoc test for significant KW data. Shoal area and acetylcholine data were analyzed using an unpaired t-test, while social interaction was assessed using the Wilcoxon-Mann–Whitney U-test. The whole-body cortisol data were analyzed by one-way ANOVA (factor: drug), followed by Dunnett's post hoc test (Experiment 1), U-test (Experiment 2) and KW test, followed by Dunn's post hoc test (Experiment 3). In all analyses, groups were compared with the control group, and *p-values* were set at <0.05. Behavioral tests were performed by highly trained experimenters in zebrafish assays, with intra/inter-rater reliability >0.9, as assessed by Spearman correlation. All data were included in analyses, without removing outliers. Statistical analyses were performed using GraphPad Prism 9.5 statistical software (GraphPad Software, San Diego, USA).

3 | Results

Experiment 1 showed significant group/treatment effects for distance traveled ($H_{5,65} = 13.05$, $p = 0.0011$), freezing frequency ($H_{5,65} = 10.63$, $p = 0.031$) and freezing duration ($H_{5,65} = 12.08$, $p = 0.0168$), as zebrafish treated with 1000 μg/L β-phenylethylamine demonstrated longer freezing compared to the control group ($p = 0.0412$), reflecting anxiety-like behavior (Figure 1). There were also significant treatment effects for time in top ($H_{5,65} = 17.99$, $p = 0.0012$), as zebrafish treated with 12 μg/L of this drug demonstrated longer time in top than the control ($p = 0.0064$), indicative of an anxiolytic-like

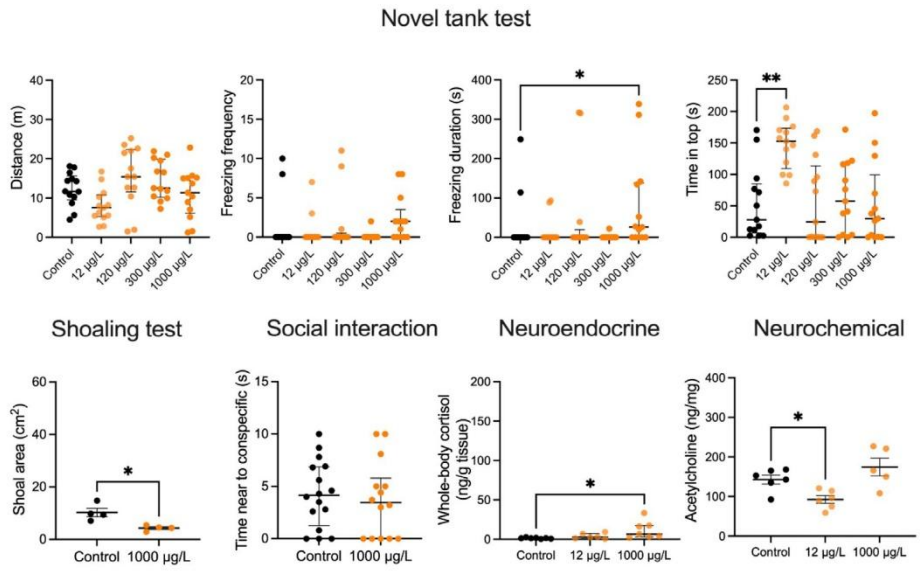


FIGURE 1 | Effects of acute 1-h β-phenylethylamine exposure on zebrafish behavior in the 6-min novel tank test (top panel, $n = 13$ per group), shoaling ($n = 4$ per cohort) and social interaction tests (control $n = 16$ and β-phenylethylamine $n = 14$), as well as whole-body cortisol levels (control and β-phenylethylamine 1000 μg/L $n = 7$, and 12 μg/L $n = 6$), and brain acetylcholine (control and β-phenylethylamine 12 μg/L $n = 6$, and 1000 μg/L $n = 5$, bottom panel). The individual behavioral data are presented as median ± interquartile range, and shoaling test, acetylcholine, and whole-body cortisol data as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, Dunn's post hoc test for significant Kruskal-Wallis data or Dunnett test for significant one-way ANOVA data.

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effect (Figure 1). Zebrafish exposed to β-phenylethylamine at 1000 μg/L also showed reduced shoal area ($p = 0.0121$), with no significant effect of this drug on social interaction ($p = 0.64$, NS). There were also significant treatment effects for whole-body cortisol levels ($F_{2,17} = 4.19$, $p = 0.0332$) and brain acetylcholine levels ($F_{2,14} = 8.03$, $p = 0.0048$), as zebrafish treated with 1000 μg/L increased the whole-body cortisol versus the control group ($p = 0.0235$), whereas 12 μg/L also decreased brain acetylcholine levels ($p = 0.0435$ vs. control, Figure 1).

Experiment 2 found a significant treatment effect for time in top ($H_{4,48} = 11.38$, $p = 0.0098$), as zebrafish treated with 7 mg/L tryptamine spent more time there versus control ($p = 0.0184$, Figure 2). In contrast, there were no significant treatment effects for distance traveled ($H_{4,48} = 0.74$, $p = 0.86$, NS), freezing episodes ($H_{4,48} = 4.02$, $p = 0.25$, NS) and freezing duration ($H_{4,48} = 3.73$, $p = 0.29$, NS) in all groups tested. However, zebrafish treated with 7 mg/L tryptamine showed reduced shoal area ($p = 0.0385$) and time near the conspecifics ($p = 0.0163$), but elevated brain acetylcholine ($p = 0.013$) and whole-body cortisol levels ($p = 0.041$) compared to the control group (Figure 2).

Experiment 3 revealed a significant treatment effect for the time in top ($H_{7,91} = 20.53$, $p = 0.0022$), as zebrafish treated with 15 μg/L of tyramine ($p = 0.0104$) and octopamine at 125 ($p = 0.0066$), 500 ($p = 0.0014$) and 1500 μg/L ($p = 0.0079$) demonstrated lower time at top versus control group, representing an anxiety-like behavior (Figure 3). There were no significant effects of these drugs for distance traveled ($H_{7,91} = 8.79$, $p = 0.18$, NS), freezing episodes ($H_{7,91} = 8.66$, $p = 0.19$, NS), and freezing duration ($H_{7,91} = 8.03$, $p = 0.24$, NS). We also found a significant treatment effect for whole-body cortisol levels ($H_{5,40} = 17.44$, $p = 0.0016$), as zebrafish treated with 15 μg/L of tyramine ($p = 0.0002$) and octopamine at 125 ($p = 0.0145$), 500 μ ($p = 0.0363$) and 1500 μg/L ($p = 0.0310$) increased the whole-body cortisol levels compared to control group (Figure 3). In contrast, there were no significant effects of these drugs on brain acetylcholine levels ($H_{5,25} = 5.51$, $p = 0.24$, NS).

4 | Discussion

The present study is the first report of overt behavioral, neuroendocrine, and neurochemical (cholinergic) effects in zebrafish following acute exposure to four major trace amines. We found dose-dependent CNS effects of acute β-phenylethylamine, at low concentrations causing anxiolytic-like effects in adult male zebrafish. Consistent with this, β-phenylethylamine in rodents ameliorates depression-like and other CNS deficits (e.g., decreased hippocampal brain-derived neurotrophic factor and TrkB expression) evoked by corticosterone injections (Lee et al. 2020). Treating with β-phenylethylamine or its precursor L-phenylalanine also improves mood in depressed patients (Sabelli et al. 1989; Sabelli and Javaid 1995). In contrast, a high concentration of β-phenylethylamine (1000 μg/L) increased anxiety-like behavior in zebrafish, prolonging freezing duration in the novel tank test and decreasing the shoal area in the shoaling test—both phenotypes associated with anxiety-like states

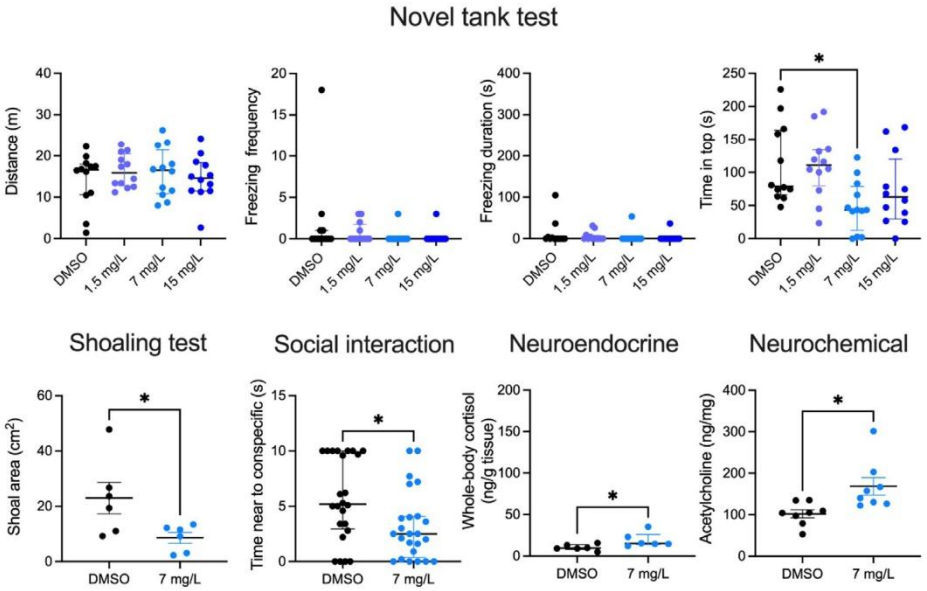


FIGURE 2 | Effects of acute 1-h tryptamine exposure on zebrafish behavioral responses in the novel tank ($n = 12$ per group), shoaling ($n = 6$ per cohort) and social interaction tests ($n = 24$). Individual behavioral and whole-body cortisol data ($n = 6$) are presented as median ± interquartile range, and shoaling test- and brain acetylcholine data ($n = 8$) as mean ± SEM. *$p < 0.05$, Dunn's post hoc test for significant Kruskal-Wallis data or *U*-test, where applicable.

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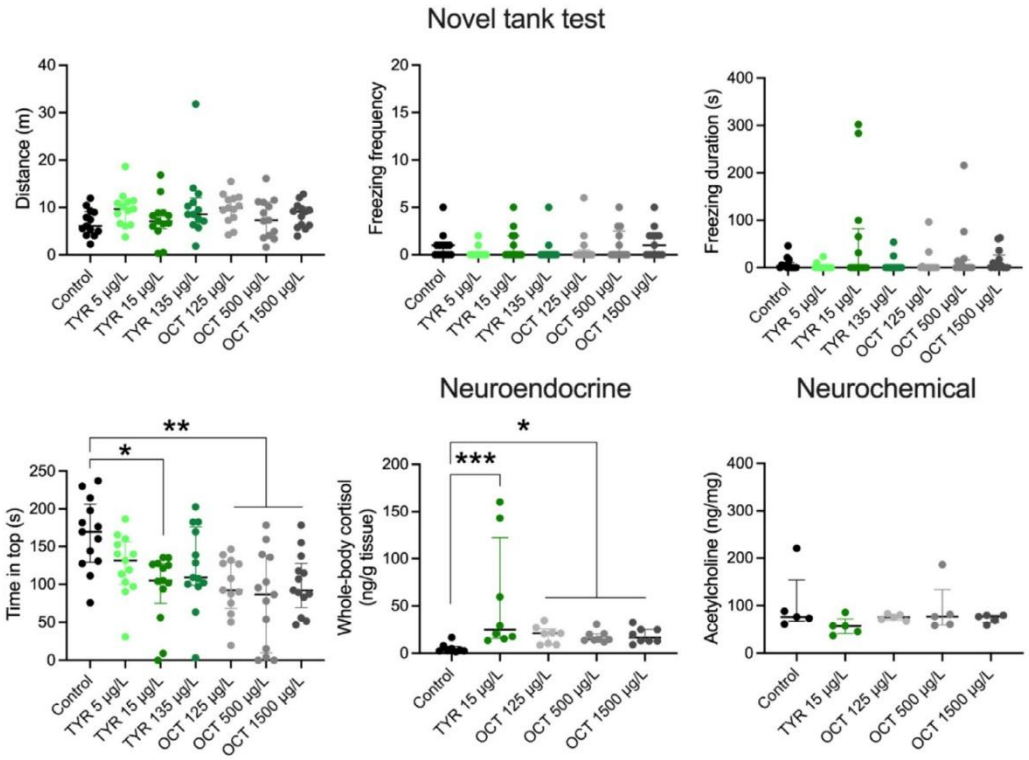


FIGURE 3 | Effects of acute 1-h tyramine and octopamine exposure on zebrafish behavioral responses in the novel tank test ($n = 13$ per group), whole-body cortisol ($n = 8$), and brain acetylcholine levels ($n = 5$). Data are presented as median ± interquartile range. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ Dunn's post hoc test for significant Kruskal-Wallis data.

(Egan et al. 2009; Kalueff et al. 2013). Interestingly, the inactivation of the MAO B gene in mice increases brain levels of β-phenylethylamine, and *MAOB*-deficient mice display increased stress reactivity (Grimsby et al. 1997), further supporting the role of β-phenylethylamine in stress response. Acute treatment with 10 mg/kg β-phenylethylamine is similarly anxiogenic in the mouse elevated plus-maze (Lapin 1993) and light/dark box tests, also reducing social interaction (Lapin 1990) and promoting stereotypies (Ryu et al. 2021).

Moreover, similar biphasic effects have been demonstrated in zebrafish by various other CNS drugs, as the exposure to intermediate concentrations of alcohol (0.5%) exerts a stimulant effect manifesting as a slight elevation of swim speed, a robust increase in turning, temporal variability in swim speed and turning, and diminished frequency of staying immobile, while high concentrations (1%) elicit an opposite, sedative effect in zebrafish larvae (Tsang et al. 2019). Butaclamol (a dopaminergic antagonist) increases larval locomotion at low-to-intermediate concentrations and reduces it at high concentrations (Irons et al. 2013). Similarly, adult fish display a U-shaped dose–response pattern in locomotor activity when exposed to ethanol (Gerlai et al. 2000), and in whole-body cortisol levels when exposed to diazepam (de Abreu et al. 2014).

Furthermore, we also found anxiogenic-like and anti-social effects for acute tryptamine (7 mg/L) in zebrafish tested here. While intravenously administered tryptamine induces serotonin toxicity syndrome-like head-weaving and hindlimb abduction in mice (Yamada et al. 1987), rats treated with *N*,*N*-dimethyltryptamine, a hallucinogenic tryptamine derivative, display similar anxiogenic-like responses in the open field test, but not in the social interaction test (Cameron et al. 2018). Acute tyramine (15 µg/L) and octopamine (125, 500, and 1500 µg/L) also evoked overt anxiogenic-like effects in male zebrafish here. Tyramine is a vasoactive amine that can trigger a migraine attack in susceptible individuals (Costa and Glória 2003), elevate blood pressure nociception, nausea, visual deficits (Astudillo et al. 2024), sympathetic activation, and, occasionally, anxiety symptoms clinically (Ogedegbe et al. 2008; Lim et al. 2021; McCabe 1986). Octopamine has also been shown to modulate CNS and behavior, and its intraventricular infusions cause various neurological deficits in rodents, also decreasing brain norepinephrine, dopamine, and serotonin levels (Chance et al. 1985).

Analyzing fish neurochemical profiles, we found that a low 12 µg/L concentration of β-phenylethylamine reduces brain acetylcholine levels (Figure 1). Interestingly, in rats this drug stimulates striatal acetylcholine release through the activation of the

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α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) glutamatergic signaling (Ishida et al. 2005). In contrast, tryptamine at 7 mg/L increased brain acetylcholine levels versus control fish here, suggesting that lower brain acetylcholine may correlate with anxiolytic- and higher levels—with anxiogenic-like behaviors (Figures 1 and 2). Notably, higher acetylcholine brain levels are associated with anxiety and depression in both rodent (Mineur et al. 2013) and human studies (Saricicek et al. 2012). Increasing brain acetylcholine levels by another acetylcholinesterase inhibitor, physostigmine, triggers anxiety, and depression in control human subjects and in patients with mood disorders (Janowsky and Overstreet 1990; Risch et al. 1980), whereas blocking acetylcholinesterase activity induces depression/anxiety-like behavior and social stress in rodents as well, and these effects can be reversed by blocking cholinergic receptors (Mineur et al. 2013). Linking the hyperactive cholinergic system to stress-related behaviors (Mineur and Picciotto 2010), our present zebrafish findings suggest that trace amines contribute to these CNS effects by modulating central cholinergic neurotransmission.

Finally, we also demonstrated that acute exposure to β-phenylethylamine (1000 μg/L), tryptamine (7 mg/L), tyramine (15 μg/L) and octopamine (125, 500, and 1500 μg/L) increased whole-body cortisol levels in zebrafish. In male rats, 10-day treatment with β-phenylethylamine elevates plasma corticosterone responses to stress (Kosa et al. 2000), and in Rhesus monkeys, intravenous administration of tryptamine increases plasma cortisol levels (Murphy et al. 1985), supporting the likely evolutionarily conserved link of trace amines to neuroendocrine (glucocorticoid) signaling.

However, the study presents several limitations and potential lines for further research. For example, we did not focus on sex differences in trace amine CNS effects, and this factor merits further scrutiny, as female zebrafish are more anxious (Genario et al. 2020) and may also differ in CNS drug responses from males (dos Santos et al. 2021). Testing a wider range of doses and treatment times (e.g., using both shorter and longer treatments than used here) may also be necessary. We did not explore trace amine effects on the brain monoaminergic (serotonin, dopamine, and noradrenaline) system that plays an important role in CNS regulation and overlaps with the former. Likewise, modulation of other neurotransmitters (including glutamate and gamma aminobutyric acid) may be interesting to assess following trace amine treatment in zebrafish models. Another interesting aspect to consider in future zebrafish studies is to compare several methods of drug administration, including both central and peripheral routes. For example, using more precise and targeted intracerebroventricular (vs. intravenous or intraperitoneal) drug administration may be interesting to perform to complement present studies using water immersion delivery of trace amines. Interestingly, a novel method of intranasal delivery of CNS drugs to adult zebrafish (Galstyan, Kolesnikova, et al. 2025) can be particularly promising to apply, given the well-reported role of various TAARs as olfactory receptors in the brain. Other limitations of the present study include its main focus on anxiety versus other neurobehavioral domains, such as motor and cognitive aspects, which clearly warrant further scrutiny. Likewise, testing individual differences in fish CNS responses to trace amines, as well as potential strain differences (e.g., especially given mounting evidence of strain behavioral differences in zebrafish assays (van den Bos et al. 2020)), will be relevant.

In addition to acute CNS effects, chronic effects of trace amines also merit further studies. For example, acute treatment with full TAAR1 agonists (e.g., RO5256390) inhibits rat serotonin and dopamine neurons, while chronic treatment increases their excitability in some brain regions (Grinchii et al. 2022). We also did not explore the effect of other trace amines and their metabolites, as well as their action on specific TAARs (e.g., on TAAR1 and other TAARs expressed in zebrafish (Li et al. 2015)), which can help understand which receptors activated by trace amines mediate anxiolytic and anxiogenic-like CNS effects. Additional putative activity of the four trace amines at other TAARs, non-TAAR receptors also warrants further studies. Furthermore, as we examined whole-brain neurochemistry, more nuanced data can be generated from region-specific analyses of the zebrafish brain.

Taken together, our findings support the importance of trace amines in the regulation of CNS and complex behaviors, and suggest the evolutionary conservation of trace amine neurobehavioral effects across taxa, from zebrafish to mammals (Galstyan, Krotova, et al. 2025). Emphasizing the growing value of zebrafish models for investigating trace amines and TAARs, this highlights the need for further translational cross-taxon research in this field.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data sets generated during and/or analyzed for the reported study are available from the corresponding authors upon reasonable request.

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5 CONCLUSÃO

O estudo permitiu ampliar a compreensão sobre os efeitos da exposição aguda às aminas-traço (β-feniletilamina, triptamina, tiramina e octopamina) em comportamentos relacionados à ansiedade em peixes-zebras, evidenciando respostas distintas entre as aminas avaliadas. A β-feniletilamina apresentou um perfil bifásico, uma vez que, a menor concentração avaliada, promoveu efeitos ansiolíticos acompanhados pela redução dos níveis de acetilcolina cerebral, enquanto, na maior concentração testada, induziu aumento do comportamento semelhante à ansiedade e dos níveis de cortisol corporal total. A exposição aguda à concentração intermediária de triptamina induziu comportamento ansiogênico, reduziu a preferência social e o comportamento de cardume e aumentou os níveis de acetilcolina cerebral e cortisol corporal total. A tiramina, na concentração intermediária, e a octopamina, em todas as concentrações testadas, também produziram efeitos ansiogênicos associados ao aumento do cortisol corporal, sem alterações nos níveis de acetilcolina cerebral. Dessa forma, os achados demonstram que as aminas-traço clássicas apresentam efeitos distintos sobre parâmetros comportamentais, neuroquímicos e endócrinos em peixes-zebras, contribuindo para ampliar a compreensão sobre a participação dessas moléculas na modulação de processos relacionados à ansiedade e à sociabilidade. Além disso, os achados reforçam o potencial do peixe-zebra como modelo experimental translacional para a investigação dos mecanismos neurobiológicos da ação das aminas-traço no SNC.

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ANEXO A - Carta de Aprovação da CEUA

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Projeto de Pesquisa

Número do projeto: 816/2024

Título: Análise do efeito de aminas traço sobre estresse e isolamento social: abordagem comportamental, monoaminérgica, imunológica e endócrina em zebrafish

Pesquisador(a) Responsável: Murilo Sander De Abreu

Situação do Projeto: PROPOSTA APROVADA

Vigência: 13/05/2024 a 01/03/2026

Fomento aprovado: NÃO

Área de conhecimento: Ciências da Saúde

Vinculação do Projeto:

- Programa de Pós-Graduação em Ciências da Saúde

Instituições Parceiras:

Nome Instituição	Origem
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE	Nacional

Membros da Equipe:

Nome	Instituição	Tipo de Vínculo	E-mail
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Tiago Franco De Oliveira	UFCSPA - UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE	Pesquisador	oliveira@ufcspa.edu.br

Declarações:

Declaro que o referido projeto não se enquadra nos termos da Resolução 466/12 do CONEP e Resolução 510/16 sobre ética na pesquisa na área de Ciências Humanas e Sociais, que regulam CEP e CONEP (obrigatório sim ou anexo do comprovante de aprovação).*	SIM

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

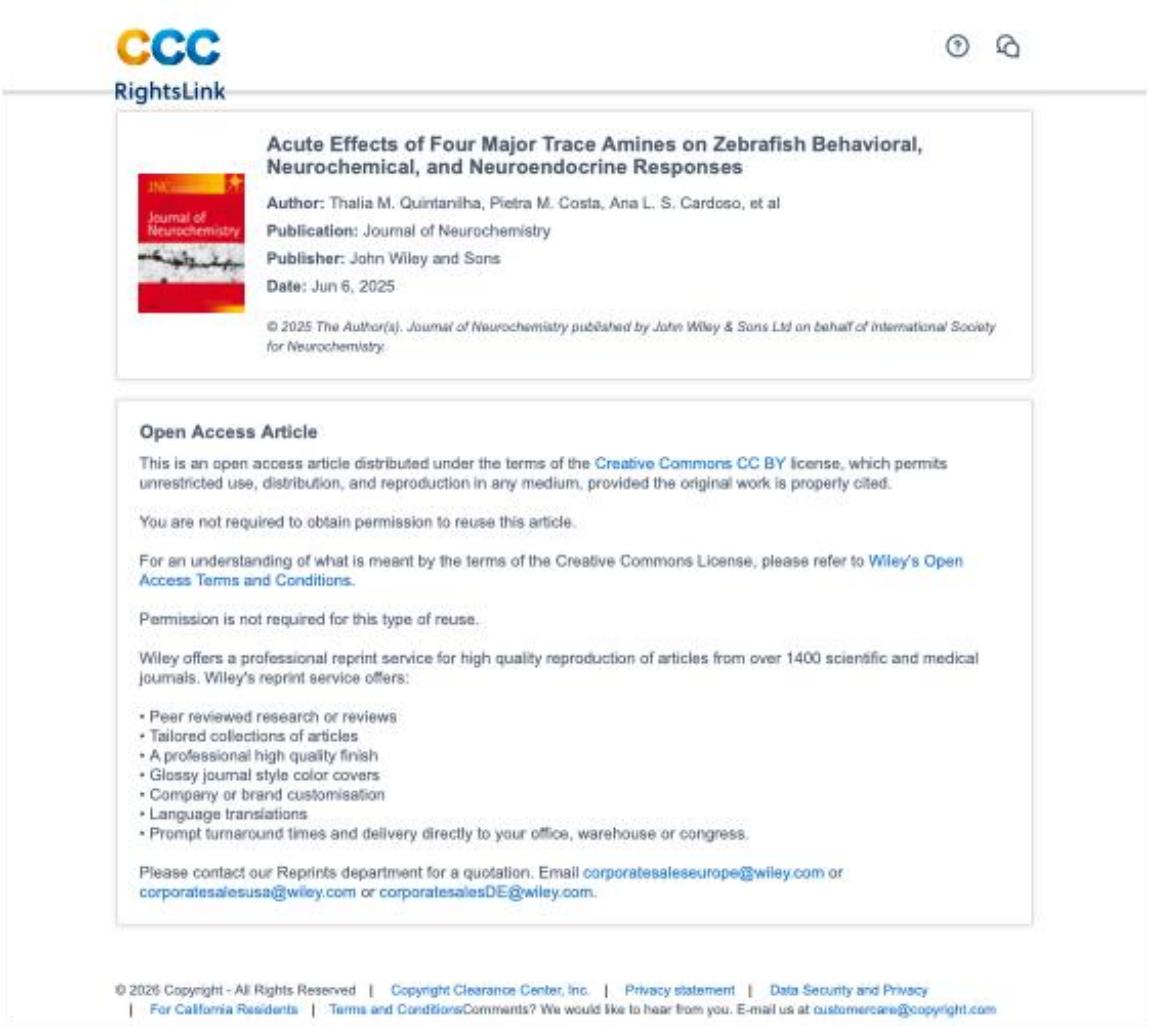
Declaro que o desenvolvimento do projeto não representa risco para os pesquisadores ou para o meio ambiente.	SIM
Declaro estar ciente de que o registro deste projeto nos respectivos órgãos (CEP, CEUA OU ComPesq) não garante a concessão de recursos financeiros por parte da UFCSPA ao projeto.	SIM
Comprometo-me a informar ao órgão que aprovou a pesquisa (CEP, CEUA OU ComPesq) alterações no projeto aprovado, tais como cronograma, financiamento e eventual cancelamento do projeto.	SIM
Declaro que a realização deste projeto poderá gerar registro de propriedade intelectual. Em caso afirmativo, o projeto será encaminhado para o NITE-Saude para o devido acompanhamento.	SIM

Declaro que o referido projeto faz uso de animais e se enquadra nos termos da Lei nº 11.794 de 8 de outubro de 2008, que regula a CEUA (obrigatório sim ou anexo do comprovante de aprovação).	SIM

Animais:

Animal/Espécie/Linhagem	Sexo	Idade	Peso	Origem	Qtd.	Data Inclusão	Data Aprovacão
peixe zebra Danio rerio selvagem	F	6 M	1 g	Delphis Aquários Atacado e Varejo ltda	562	15/04/2024	22/05/2024
peixe zebra Danio rerio selvagem	M	6 M	1 g	Delphis Aquários Atacado e Varejo ltda	562	15/04/2024	22/05/2024

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Acute Effects of Four Major Trace Amines on Zebrafish Behavioral, Neurochemical, and Neuroendocrine Responses

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