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Beyond Behavioral Observation: Digital Biomarkers and Precision Sedation for Non-Verbal Dental Patients: Narrative Review

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Abstract

Behavioral assessment scales are widely used to evaluate dental anxiety in clinical practice; however, they have important limitations because they rely on subjective interpretation and may not accurately identify anxiety in non-verbal dental patients, including individuals with developmental disabilities and anxious pediatric patients with limited communication abilities. Recent advances in objective physiological monitoring and digital technologies offer promising alternatives for individualized anxiety assessment and precision sedation in this vulnerable population. This review examines whether objective digital biomarkers can overcome the limitations of behavioral assessment in non-verbal dental patients and how close these technologies are to clinical application in precision sedation. This narrative review synthesizes evidence on physiological biomarkers, including heart rate variability, electrodermal activity, pupillometry and respiratory variability, as well as computer vision, multimodal biosensing and closed-loop monitoring systems. We retrieved relevant literature from PubMed, Scopus and Google Scholar, prioritizing studies published from 2019 onward. Current evidence indicates that objective physiological biomarkers, computer vision and multimodal wearable biosensing provide complementary information for anxiety assessment in non-verbal dental patients. Integrating these digital biomarkers with artificial intelligence enables individualized monitoring and establishes the foundation for precision, closed-loop sedation; however, further clinical validation and standardization remain necessary. No single technique is sufficient on its own; rather, a multimodal, patient-specific approach is needed to improve the accuracy and safety of anxiety management in special care dentistry, particularly in populations for whom conventional behavioral observation is limited or unreliable.

Keywords: Heart Rate Variability; Electrodermal Activity; Computer Vision; Closed-Loop Sedation; Wearable Biosensors; Special Care Dentistry

Introduction

Pain represents a multidimensional sensory and emotional phenomenon linked to actual or potential tissue injury [1]. Within the dental context, anxiety is defined by apprehension regarding painful stimuli, sensory triggers and perceived loss of autonomy [3,5]. These factors pose significant obstacles to oral healthcare, leading to procedural avoidance, clinical interruptions and psychological distress for both providers and patients. Patients who lack verbal communication abilities are often marginalized by traditional diagnostic frameworks, elevating the probability of undetected suffering and suboptimal intervention [1]. This issue is particularly acute among individuals with neurodevelopmental disorders, whose communication barriers may mask the presence of significant pain and anxiety [2]. Pediatric dental anxiety is a complex, multifactorial state shaped by individual psychology, developmental maturity, parental influence and the clinical environment; consequently, anxious children face an increased burden of untreated disease and often require more extensive therapeutic measures [3].

Strategic management of dental apprehension is vital to optimize clinical outcomes and foster sustainable, positive attitudes toward oral health. While pharmacological interventions, such as sedation or general anesthesia, provide necessary support in specific scenarios, they fail to modify underlying psychological drivers or facilitate the acquisition of functional coping mechanisms [2]. Thus, non-pharmacological modalities remain foundational to pediatric dentistry; behavioral guidance, communication protocols and environmental adaptations are frequently employed to mitigate fear, enhance predictability and bolster the patient's sense of agency during care [3].

In contemporary practice, behavioral instruments such as the Frankl Scale and the Venham Scale are routinely utilized to monitor pediatric responses during treatment, while self-report tools like the Visual Analog Scale (VAS) track perceived distress [4]. Despite their widespread use, these metrics are inherently subjective, relying on the clinician's interpretation; moreover, their reliability diminishes in non-verbal populations where observable behavior may not accurately reflect the internal autonomic state [5]. These constraints underscore the need for objective diagnostic alternatives that can augment behavioral observation and refine assessment in patients with limited communication capacity.

Emerging research has investigated physiological indices, including Heart Rate Variability (HRV) and Electrodermal Activity (EDA), as quantifiable markers of emotional arousal during dental procedures [3]. While initial findings are encouraging, the current literature is constrained by modest cohort sizes and the frequent exclusion of patients exhibiting profound behavioral dysregulation. Nevertheless, integrating physiological biomarkers with conventional behavioral data may improve the precision of anxiety monitoring and support the transition to more personalized management strategies [6].

Physiological Biomarkers of Anxiety

Behavioral assessment scales remain the primary approach for evaluating dental anxiety and rely on external interpretation; however, their consistency is limited in non-verbal individuals and patients with neurodevelopmental disorders [2]. Anxiety reliably activates the autonomic nervous system, increasing heart rate, blood pressure and respiratory rate while producing measurable, quantifiable physiological responses that can complement or replace subjective behavioral assessment [5].

Heart Rate Variability (HRV), reflecting the balance between sympathetic and parasympathetic tone, is among the most extensively studied biomarkers. Its prominence rests on three practical grounds: autonomic imbalance is one of the earliest and most consistent physiological correlates of anxiety; HRV can be derived non-invasively from electrocardiographic or photoplethysmographic signals already captured by routine monitoring and consumer wearables; and its time- and frequency-domain indices are standardized, which permits comparison across studies and devices. [6] A large meta-analysis of 99 case-control studies (n = 10,456) demonstrated that resting-state HRV is significantly reduced across anxiety disorder subtypes, including generalized anxiety, panic disorder and social anxiety disorder, compared with healthy controls, with the strongest effect observed in generalized anxiety disorder (Hedges' g = -0.64). [6] Notably, HRV reactivity to acute stressors did not reliably differentiate anxious from non-anxious individuals, suggesting that resting-state measurements may hold greater diagnostic value than task-induced changes [6,7]. Complementary experimental data support this autonomic signature: video-induced anxiety in healthy adults produced significant reductions in HRV time-domain indices, including SD, RMSSD and SDNN, within minutes of stimulus exposure [8]. However, when HRV is monitored via commercial devices, associations with anxiety symptoms become inconsistent, highlighting that population characteristics substantially affect its clinical utility [9].

Electrodermal Activity (EDA), a direct index of sympathetic sudomotor activation, offers rapid-onset sensitivity to emotional arousal through Skin Conductance Responses (SCR) [9]. Because standard EDA acquisition sites on the fingers and palms can be inaccessible when hands are occupied or restrained during clinical procedures, researchers have investigated alternative body locations. Chest placement demonstrated the strongest correlation with finger-based recordings across both tonic and phasic components, whereas forehead placement performed poorly in short-duration recordings, likely due to lower eccrine gland density [10]. These findings have plausible implications for pediatric or special-needs dental contexts, where unobtrusive sensor placement is often required.

Pupillometry provides an additional, non-contact window into autonomic arousal via Pupillary Reflex Dilation (PRD), mediated by locus coeruleus activation [10]. In healthy volunteers, PRD increased significantly during painful heat stimulation relative to baseline and decreased proportionally following endogenous pain modulation, paralleling but not directly correlating with subjective pain ratings [11]. The dissociation observed between objective autonomic response and subjective self-report suggests potential value for non-verbal populations, for whom pain and anxiety self-report is inherently unreliable [12].

Respiratory variability, though less studied in isolation, shows a consistent pattern of faster, shallower breathing under anxiety, with reduced Breathing-Rate Variability (BRV) time-domain indices mirroring HRV changes [7]. Collectively, no single biomarker is sufficient; each captures a distinct facet of autonomic dysregulation with varying sensitivity, latency and susceptibility to artifact [13]. Table 1 summarizes the comparative profile of the four main physiological biomarkers discussed in this section.

Biomarker	System	Sensor Type	Latency	Clinical Notes
Heart Rate Variability (HRV)	Cardiac (autonomic)	Wearable ECG / PPG	Resting state	Reduced in anxiety; resting HRV more reliable than stress-induced; commercial devices inconsistent [6,7]
Electrodermal Activity (EDA)	Sympathetic sudomotor	Finger/wrist / chest patch	Rapid (<1 s)	Chest placement best when hands occupied; high artifact sensitivity to movement [9,10]
Pupillometry (PRD)	Locus coeruleus / sympathetic	Camera-based (non-contact)	Rapid (<1 s)	Non-contact; useful in non-verbal populations; dissociates from subjective pain rating [11,12]
Respiratory Variability (BRV)	Autonomic/respiratory	Chest strap/respiration sensor	Seconds	Faster, shallower breathing under anxiety; mirrors HRV indices; less studied in isolation [7]

Table 1: Comparative profile of physiological biomarkers of dental anxiety: system, sensor type, latency and clinical considerations for non-verbal patient monitoring [6,7,9-12].

Computer Vision and AI-Based Facial and Behavioral Analysis

The assessment of anxiety, pain, discomfort and potentially inadequate sedation in non-verbal dental patients continues to depend largely on the clinical interpretation of facial expressions, vocalizations, body movements, withdrawal reactions, muscle tension and changes in cooperation [14]. These observations provide clinically essential information; however, they may be intermittent, observer-dependent and difficult to interpret, since similar behaviors may reflect pain, fear, sensory overload, emotional distress or medication-related effects. Video ethnography in dental care has shown that anxiety may manifest through postural changes, facial microexpressions, respiratory alterations, gaze shifts and hesitation during interactions, highlighting that distress is dynamic and context-dependent rather than reducible to a single visible sign [2]. Computer vision and artificial intelligence could complement clinical observation by transforming these behavioral manifestations into quantifiable digital features [15].

Automated facial analysis uses facial detection, feature extraction and machine-learning algorithms to identify patterns in facial expressions and microexpressions. Recent systematic evidence indicates that artificial intelligence, particularly convolutional neural networks, transfer-learning techniques and hybrid deep-learning approaches, can identify facial patterns associated with various neurological and psychological conditions, including anxiety and autism [11,16]. An automated facial recognition system based on deep learning has also been applied to pain assessment in adults with cerebral palsy; a population in which limited self-reporting and altered facial expression patterns present challenges comparable to those found in non-verbal dental patients. The system demonstrated technical feasibility for recognizing pain-related facial features without relying on verbal self-report

[12]. Similarly, artificial intelligence models using facial images have shown promising results in assessing pain at different intensity levels, suggesting that visible facial information may provide relevant objective data for recognizing distress when verbal self-report is limited [13]. However, these findings cannot yet be directly extrapolated to dental anxiety or sedation depth and further research is needed to validate their applicability in these settings [17]. Fig. 1 outlines the sequence of processing steps by which video of a patient's face is converted into quantified behavioral variables, together with the point at which the evidence currently stops.

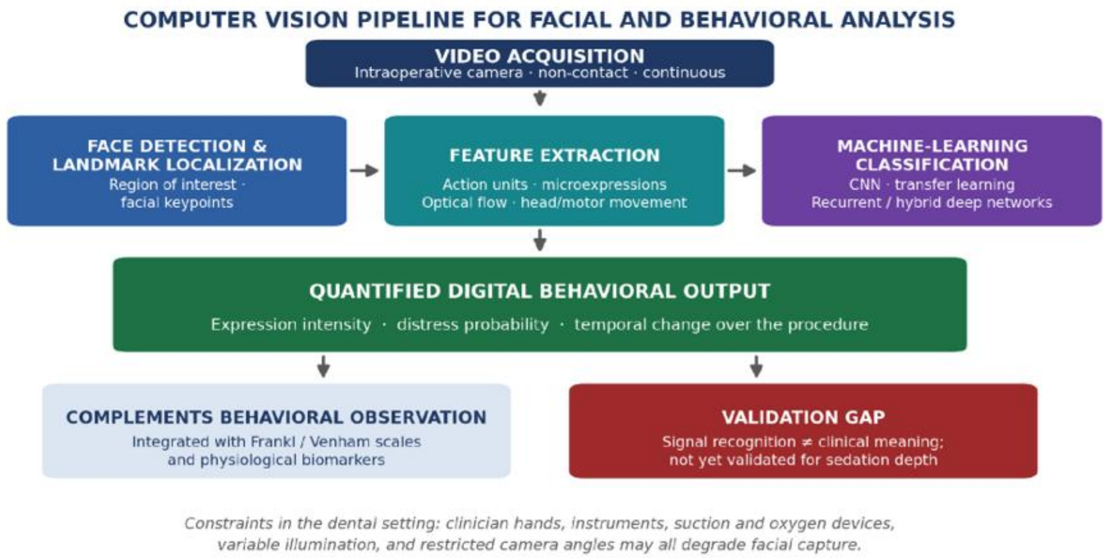

Figure 1: Computer vision pipeline for facial and behavioral analysis in non-verbal dental patients: acquisition, feature extraction, classification and the validation gap between signal recognition and clinical meaning [11-16,18,19].

Facial expression alone captures only part of the behavioral picture and computer vision becomes more informative once temporal and motor information are added. Where a static frame records a single configuration of the face, sequential analysis tracks how that configuration changes as a procedure unfolds. Deep-learning work on automatic pain assessment supports this: models that combine modalities and analyze data as a sequence outperform those trained on one modality or on isolated images [14]. Movement carries comparable information. Facial landmark tracking, optical flow, appearance modeling and deep neural networks have each been used to detect and measure orofacial movement with a precision that unaided observation cannot match [15,18]. What these methods share is a common function: they convert transient, easily missed physical activity into continuous numerical variables. That conversion makes the approach relevant to non-verbal dental patients, since it provides a behavioral record that does not depend on the clinician noticing a cue when it occurs [19].

Recent prospective evidence has specifically evaluated computer vision during procedural sedation. Zarghami, et al., used facial video recordings during interventional radiology procedures to investigate automated pain detection, demonstrating the feasibility of continuous computer-vision assessment in sedated patients [16]. This represents an important step toward clinical application because it evaluates facial analysis under conditions in which medications, procedural stimulation and limited communication ability may alter observable behavior [20]. However, successful recognition of a facial or behavioral pattern does not necessarily establish its clinical meaning; a grimace or withdrawal movement may represent pain, anxiety, sensory discomfort, voluntary resistance or an involuntary response [21]. In dental practice, technical performance may be further affected by the clinician's hands, instruments, suction equipment, oxygen-delivery devices, changes in illumination and limited camera angles. Table 2 summarizes the main computer vision and AI approaches reviewed in this section.

Approach	Algorithm Type	Key Finding	Key Reference
Automated facial expression analysis	CNN; transfer learning	Identifies anxiety, pain, autism-related patterns; not yet validated in dental settings	Ghafarfaraji, 2026 [11]; Huo, et al., 2024 [13]
Deep learning pain assessment	Recurrent/hybrid deep networks; sequential video	Multimodal approaches outperform single-frame; temporal context improves accuracy	Gkikas and Tsiknakis, 2023 [14]
Facial recognition in non-verbal patients	CNN on pain image databases (cerebral palsy)	Technical feasibility without self-report ability demonstrated	Sabater-Gárriz, et al., 2024 [12]
Orofacial movement quantification	Optical flow; landmark detection; appearance models	Captures head withdrawal, muscle tension, repetitive movements; dental applicability theoretical	Tufano, et al., 2022 [15]
CV during procedural sedation	Video analysis during interventional radiology	Feasibility of continuous CV in sedated patients demonstrated; closest to dental context	Zarghami, et al., 2026 [16]

Table 2: Summary of computer vision and artificial intelligence approaches for facial and behavioral analysis: algorithm type, key findings and primary evidence sources.

Multimodal Biosensing and Wearable Technologies

While individual physiological biomarkers provide valuable insights into autonomic responses associated with dental anxiety, emotional states are inherently multidimensional and cannot be fully characterized by a single physiological parameter [22]. Consequently, recent advances have shifted toward multimodal biosensing, in which wearable technologies continuously acquire complementary physiological signals and integrate them to provide a more robust representation of the patient's emotional and autonomic state [23]. This approach reduces the limitations of isolated biomarkers and establishes the technological foundation for precision sedation by enabling continuous, objective, patient-specific monitoring throughout dental treatment [17,18].

Modern wearable devices simultaneously collect multiple biosignals, including Electrocardiography (ECG), Photoplethysmography (PPG), Heart Rate Variability (HRV), Electrodermal Activity (EDA), respiratory patterns, skin temperature and body motion [25]. Rather than treating each variable independently, multimodal platforms combine these complementary measurements to capture the complex interactions among sympathetic activation, cardiovascular regulation, respiratory dynamics and peripheral autonomic responses that accompany anxiety [26]. Recent systematic evidence demonstrates that multimodal approaches consistently outperform single-sensor systems for anxiety detection, highlighting the clinical value of integrating heterogeneous physiological signals into unified analytical models [17]. Fig. 2 illustrates how these separate signal streams are conditioned, fused and modeled to yield a single composite biomarker.

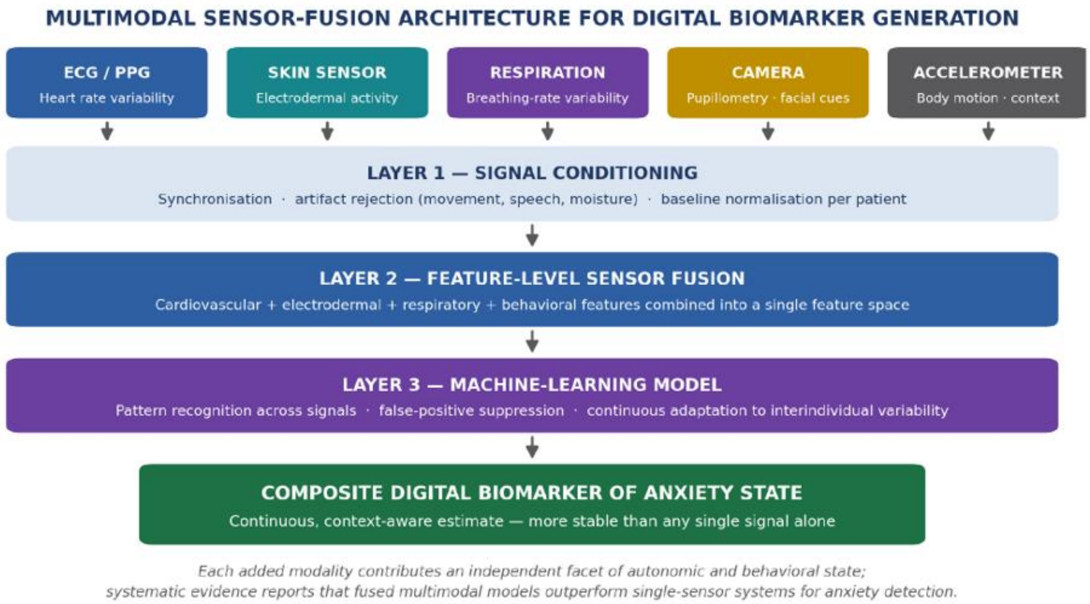

Figure 2: Multimodal sensor-fusion architecture: independent physiological and behavioral signals are conditioned, combined at the feature level and processed by machine-learning models to generate a composite digital biomarker of anxiety state [17,19-22,25,26].

Miniaturization of biosensors has enabled wearable technologies capable of unobtrusive monitoring during routine dental care [27]. Wrist-worn smartwatches, finger sensors, adhesive skin patches, chest straps and wireless photoplethysmographic devices now permit continuous physiological acquisition without substantially interfering with patient comfort or clinical procedures. In dentistry and oral and maxillofacial applications, biosensor technologies are increasingly being explored not only for physiological monitoring but also to support individualized patient management through real-time digital health ecosystems; these systems create opportunities to integrate physiological information directly into the clinical workflow while minimizing interruptions to treatment delivery [18,19]. Table 3 presents a comparative overview of wearable device types and their applicability in the dental setting.

Device Type	Signals Captured	Advantages	Limitations in Dental Use	Signal Quality
Smartwatch/wristband	PPG, HRV, skin temperature	Non-invasive; accepted by patients	Motion artifact; no EDA in most devices	Moderate
Adhesive skin patch	ECG, EDA, respiration	Continuous; minimal interference	Adhesion failure; moisture sensitivity	Moderate to High
Finger sensor	PPG, SpO2, HRV	High signal quality	Occupies finger (dental access issue)	High
Chest strap	ECG, respiration, EDA (chest)	Best alternative EDA site; validated	Requires setup; patient cooperation needed	High
Camera-based (non-contact)	Pupillometry, facial cues	No sensor contact needed	Lighting variability; camera angle; occlusion by instruments	Experimental

Table 3: Comparative overview of wearable biosensor platforms: signals captured, advantages, limitations in dental use and signal quality assessment [17,18].

The greatest strength of multimodal biosensing lies not simply in collecting larger quantities of physiological data but in integrating these data through artificial intelligence and machine learning algorithms. Sensor-fusion approaches combine cardiovascular, electrodermal, respiratory and behavioral information to generate digital biomarkers that more accurately reflect the dynamic nature of anxiety than any individual signal alone [19,20]. Machine learning models further enhance this process by recognizing complex physiological patterns, reducing false-positive detections caused by movement artifacts or isolated autonomic fluctuations and adapting continuously to interindividual variability [17,21,22]. Such multimodal frameworks therefore support a transition from intermittent behavioral assessment toward continuous, context-aware physiological monitoring capable of guiding personalized clinical decision-making [21]. The practical implication is a change in what a sedation record can contain: rather than a handful of observations noted at intervals, it becomes a continuous physiological trace that can be compared against the patient's baseline, reviewed after the appointment and carried forward to inform planning at the next visit [21,22].

Despite these advances, important challenges remain before multimodal wearable technologies can be routinely incorporated into dental practice. Signal quality may be affected by patient movement, speech, moisture or variations in sensor positioning; differences in hardware platforms and data-processing algorithms continue to limit interoperability across devices [23]. Additional considerations include patient privacy, secure management of continuously acquired physiological data, user acceptance and the need for standardized validation protocols across diverse clinical populations [24]. Furthermore, although wearable technologies have demonstrated promising performance in controlled research environments, prospective clinical studies conducted during routine dental treatment remain relatively limited [20,21]. Collectively, multimodal biosensing represents an essential step toward objective and individualized anxiety assessment in dentistry, providing the technological infrastructure required for the next stage of precision sedation: closed-loop systems capable of dynamically adapting sedation according to continuously monitored patient physiology [22,23].

Closed-Loop Sedation Systems with Real-Time Physiologic Feedback

Closed-loop sedation systems represent the next step after passive monitoring: instead of the clinician manually adjusting the drug infusion based on observed values, the system does so autonomously. It operates as a continuous loop in which a sensor captures the physiologic signal, an algorithm processes it and the system automatically adjusts the drug dose in real time [24]. This is particularly important for non-verbal patients, because the conventional approach of observing behavior to detect distress cues does not work reliably when the patient cannot communicate how they are actually feeling [25].

Most of what is currently known comes from Bispectral Index (BIS)-guided closed-loop propofol systems. A recent systematic review and meta-analysis pooling 17 randomized controlled trials on BIS-guided setups found that these systems are safer than manual control, as patients spend less time either too deeply sedated or under-sedated [24]. This is consistent with an earlier meta-analysis on BIS-guided Target-Controlled Infusion (TCI), which also found tighter, more consistent control of hypnotic depth than manual dosing [26].

In dentistry, the situation is more complex for non-verbal patients. A systematic review on conscious sedation for dental treatment in patients with intellectual disability found significant variability across studies in drugs, monitoring approaches and routes of administration; essentially, no standardized method exists for achieving real-time feedback and very few studies use true closed-loop systems [27]. This represents a significant gap, since this population would benefit most from an objective physiologic signal rather than relying on behavioral interpretation alone [28].

These systems are only as reliable as the pharmacokinetic model running underneath them. A study testing pediatric propofol pharmacokinetic models found that predictive accuracy varied considerably between models which represents a genuine problem for anyone trying to build a closed-loop system for children or for any patient with atypical drug handling; a poor model can lead to poor dosing decisions regardless of sensor quality [27,29]. Fig. 3 outlines the control architecture of such a system and the role of the pharmacokinetic model within it.

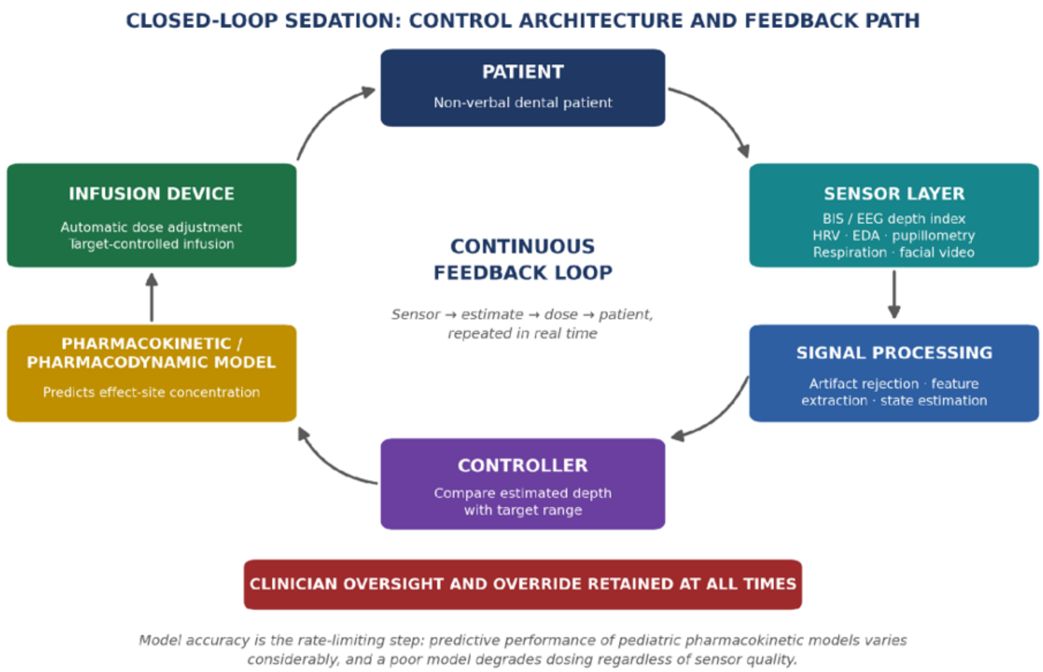

Figure 3: Closed-loop sedation control architecture, showing the continuous feedback path from sensor to dose and the pharmacokinetic model that limits its accuracy. Clinician oversight is retained throughout [24-27,29].

Evidence from adjacent fields further supports transferring closed-loop control to dental sedation. Closed-loop oxygen control and closed-loop ventilation in ICU patients consistently demonstrate that continuous feedback keeps patients better within target ranges and reduces staff workload without compromising safety [28,29]. Taken together with evidence on automated sedation and weaning protocols in critical care [30]. These findings suggest that closed-loop control represents a solid and transferable concept; one that could eventually incorporate multimodal signals such as heart rate variability, electrodermal activity or pupillometry as additional digital biomarkers for non-verbal dental sedation, rather than relying on BIS alone [31,32]. Fig. 4 maps the relative maturity of the evidence across these adjacent domains and the pathway by which it might transfer to dental practice.

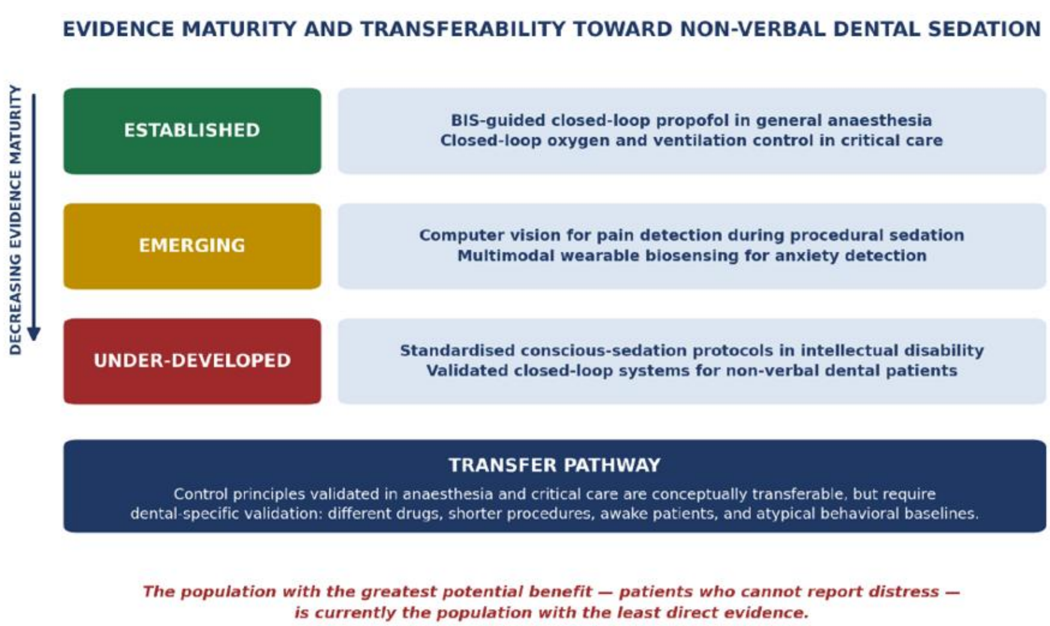

Figure 4: Evidence maturity across adjacent domains and the transfer pathway toward non-verbal dental sedation [24,26-30].

Fig. 5 presents a proposed clinical decision algorithm integrating multimodal physiological monitoring with behavioral assessment for precision sedation in non-verbal dental patients. The algorithm is intended as a clinical guide; implementers must adapt it to the specific patient, setting and available technology.

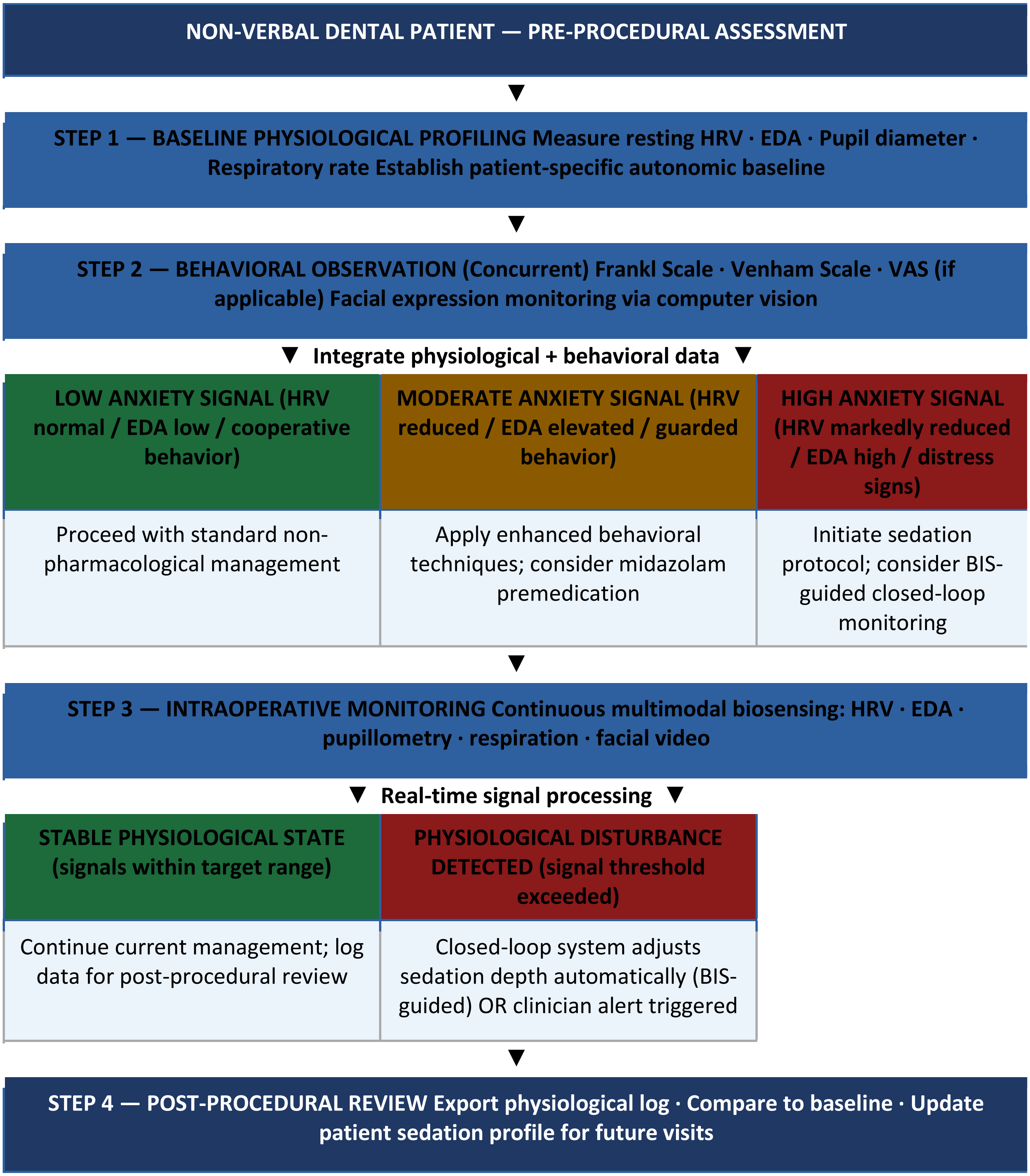

Figure 5: Proposed clinical decision algorithm for multimodal physiological monitoring and precision sedation in non-verbal dental patients. HRV: Heart Rate Variability; EDA: Electrodermal Activity; BIS: Bispectral Index; VAS: Visual Analog Scale.

Discussion

The four bodies of evidence reviewed here converge on a single observation: each modality captures a partial and complementary aspect of the anxiety response and none is sufficient in isolation. Resting heart rate variability reliably distinguishes anxious from non-anxious individuals, yet it does not capture reactivity to acute stressors and its performance degrades when acquired through commercial devices [6,7,9]. Electrodermal activity responds within seconds but depends on sensor placement that dental treatment frequently makes unavailable [9]. Pupillometry requires no contact at all and, importantly, dissociates from subjective pain report, which is precisely the property required where self-report is absent [10,11]. Computer vision contributes behavioral information that physiological sensors cannot provide, but has not been validated against sedation depth [16,17]. The consistent superiority of multimodal over single-sensor models is therefore not a surprising result; it follows from the fact that each signal describes a different facet of the same underlying state [17].

A second theme runs through the literature and deserves explicit statement: detecting a signal is not the same as knowing what it means. A grimace, a withdrawal movement or a rise in sympathetic tone may represent pain, anxiety, sensory discomfort, voluntary resistance or an involuntary reflex and the physiological trace does not distinguish among them [21]. This interpretive gap, rather than sensor performance, is the principal obstacle to clinical translation. It also explains why these technologies are better understood as instruments that enrich clinical judgment than as instruments that replace it.

The evidence base carries recognizable limitations. Most studies enroll small, heterogeneous samples and are conducted in controlled research environments rather than during routine dental treatment; prospective work in non-verbal dental populations specifically remains scarce [20,21]. The asymmetry is striking in closed-loop control, where evidence from general anesthesia and critical care is robust [24,25,28-30]. While dentistry has no standardized approach to conscious sedation in patients with intellectual disability and very few studies using true closed-loop feedback [26]. Underlying model accuracy compounds the problem, since predictive performance of pediatric pharmacokinetic models varies considerably between models and a poor model degrades dosing irrespective of sensor quality [27]. The population that stands to gain most from objective monitoring is thus the population with the weakest direct evidence.

Several implications follow for the next phase of work. Validation cohorts must include non-verbal and neurodivergent patients rather than extrapolating from adult volunteers and biomarker acquisition and reporting require standardization before results can be compared across devices and centers [17,20]. Multimodal models should be tested directly against established behavioral scales during routine treatment, so that incremental clinical value can be quantified rather than assumed. Pharmacokinetic and pharmacodynamic models need validation in pediatric and atypical populations before closed-loop control can be considered for dental sedation [27]. In parallel, governance frameworks addressing data privacy, algorithmic bias, equitable access and clinical accountability must be developed alongside the technology, not after it [22-24]. For the foreseeable future, the realistic clinical role of these systems is decision support under continuous clinician supervision, with autonomous control remaining a longer-term prospect contingent on evidence that does not yet exist.

Conclusion

The shift from subjective behavioral observation toward objective physiological monitoring is a meaningful step for patients whose distress cannot be reported. Heart rate variability, electrodermal activity, pupillometry, respiratory variability and AI-assisted facial analysis each supply continuous information that behavioral scales cannot and integrating them through multimodal wearables and closed-loop systems offers a route to sedation that adapts to the individual patient. Evidence has not yet matched that potential: current studies are small, heterogeneous and rarely conducted during routine dental care. These technologies should therefore complement clinical judgment rather than replace it, within transparent and validated decision-support systems and their advance depends on large-scale prospective studies, standardized biomarker protocols and equitable development that keeps precision sedation accessible to the patients who need it most.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Data Availability Statement
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement
This manuscript is a narrative literature review and did not involve human participants, animal subjects or patient data. No ethical approval was required.

Informed Consent Statement
Not applicable.

Authors' Contributions
All authors contributed equally to this paper.

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